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ORIGINAL ARTICLES

THE PATHOLOGY OF INFLUENZA AS SEEN IN THOSE WITH CHRONIC MENTAL DISEASE*

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A LARGE number of excellent reports with accurate descriptions of the lesions in various organs taken at autopsy on individuals dead from influenza have been written by authorities of many countries and particularly of Military General Hospitals, but as these accounts deal largely with material which was supposedly approximately healthy before the onset of the disease, it was thought expedient to study the condition when superimposed on depleted tissues which were partially disorganized by other diseases.

The following investigation was carried out on autopsy material obtained at the Government Hospital for the Insane, Washington, D. C.

This group of 42 cases is particularly interesting because (1) in most instances the acute infection occurred in tissues already considerably impaired by the chronic processes usually existant in psychopathic and neurologic patients, (2) special attention has been given to the histopathologic changes in the central nervous system; and (3) the ages of the individuals studied range from 7½ months (fetus) to 78 years, with many cases past middle life, and 5 cases over 70 years of age.

Table I is given to indicate the age, the sex, the original lesions, and the psychosis.

EXTERNAL APPEARANCES

At the examination of a body dead from influenza, one is impressed with the general picture of asphyxia; the frothy exudate from the mouth and often from the external nares, the lividity about the face, neck, and shoulders and the cyanosis of the nails; all point toward an acute asphyxiative condition. Often rigor mortis is rapid in onset and of prolonged duration.

*From the Government Hospital for the Insane, Washington, D. C.

MUCOUS MEMBRANES

The mucous membranes present two types of change which in the mouth may be present even as far as the mucocutaneous junction of the lips, which are then a striking bright red in color. In type 1 the trachea and bronchioles contain a frothy semifluid, blood tinged exudate, and the lining membranes are characterized by networks of dilated, plainly visible, bright red capillaries.

Microscopically we see the lining cells in stages of albuminous degeneration with some areas of death and desquamation. The capillaries are seen to be stretched almost to the point of rupture, being densely packed with red blood corpuscles.

The mucous membranes of the esophagus, stomach and intestines, are usually of this type; although some have spoken of the submucous hemorrhages of petechial nature, as being frequent in influenza, it should not be connected in any special way, as such reactions are noted in many diseases not particularly related. The membranes of the bladder and genital organs are also usually of the Type 1.

In Type 2 there is an intense congestion, rupturing the vessel walls, with an extensive pouring out of corpuscles producing a general diffuse, glairy, beefy red color to the structures. This variety of change is probably due to defective oxygenation of the blood, producing an acidosis which affects the stability of the vessel walls. The microscope discloses a very striking and characteristic picture. The blood vessel walls of the submucosa are under tension, the muscle fibers appear dead and unstained, the nuclei of the cells very pale, and the endothelial lining cells dislodged. Large numbers of vessels with these deficient walls have ruptured and the red blood cells have poured out into the surrounding substances, where focal necroses are produced from pressure and lack of nutrition. Endothelial leucocytes gather in the vicinities of these hemorrhages and ingest the masses of blood pigment from the disintegrated red cells. In the trachea and bronchi the epithelium may be indistinct and in places completely degenerated, there remaining only a faint outline of former epithelial structure. Submucous tissues are edematous, congested and contain many lymphocytes. The glandular areas contain numerous lymphocytes, much pigment deposit, dilated vessels, and a few acute inflammatory cells.

SEROUS SURFACES

Pleurae.—The parietal pleura presents a diffusely reddened surface over which the dilated blood-filled capillaries stand out prominently. The visceral pleura is usually darkened and frequently exhibits petechial or confluent hemorrhages.

Some have claimed that the pleura is free from exudate and is usually without fibrin deposits; but in this group of cases a thin layer of fibrinous exudate, especially between the lobes of the lungs, could practically always be demonstrated when selective staining methods were employed.

In cases of chronic pleuritis of ancient origin, new formation fibrin is pres-

ent in large amounts (see Fig. 1) and the bands of adhesion are strikingly hemorrhagic.

Due to rupture of the surface alveoli of the lungs, many air blebs occur beneath the pheurae.

The pleural fluids vary greatly in amount and character; in some instances as many as 500 c.c. of clear yellowish fluid are present, but usually the pleural cavity contains only a few cubic centimeters of blood-tinged slightly turbid fluid, which in cases associated with jaundice presents the additional bile tint.

Pericardium.—A moderate serous effusion is almost always present, with the fluid sometimes clear, but more often blood-tinged. Both layers of the pericardium are most intensively congested and occasionally a case is found presenting distinct petechial hemorrhagic spots usually distributed along the left side of the sac.

Fig. 1.—Hemorrhages and strands of fibrin in sections of chronic pleuritis.

Peritoneum.—There is an intense congestion of the entire peritoneum with all minute vessels standing out prominently. The vessels of the intestinal surfaces are frequently so engorged as to produce a contrast not unlike that effected by an exaggerated over-colored painting.

LUNGS

Because of the prevalence of chronic pulmonary conditions, particularly the secondary indurative pneumonias, in this class of individuals, the lung findings are not easily arranged into the fairly distinct types usually described in connection with this disease; however, the acute inflammatory edema or hemorrhagic pneumonitis is easily demonstrated in those dying shortly after the onset of the disease. In these cases, regardless of the type or extent of chronic condition present, about 90 per cent of the lung tissue is involved

in a process characterized by a rounded dark red, firm, edematous surface without lobular distribution.

An enormous accumulation of bloody fluid is present in all portions, and on section streams of this fluid often to the amount of 100 to 200 c.c. pour spon-

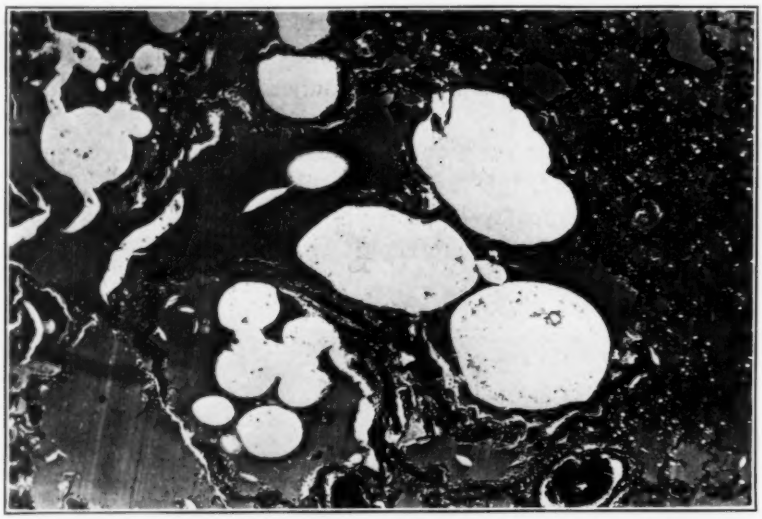

Fig. 2.—Acute inflammatory pulmonary edema with alveoli widely distended with acellular fluid.

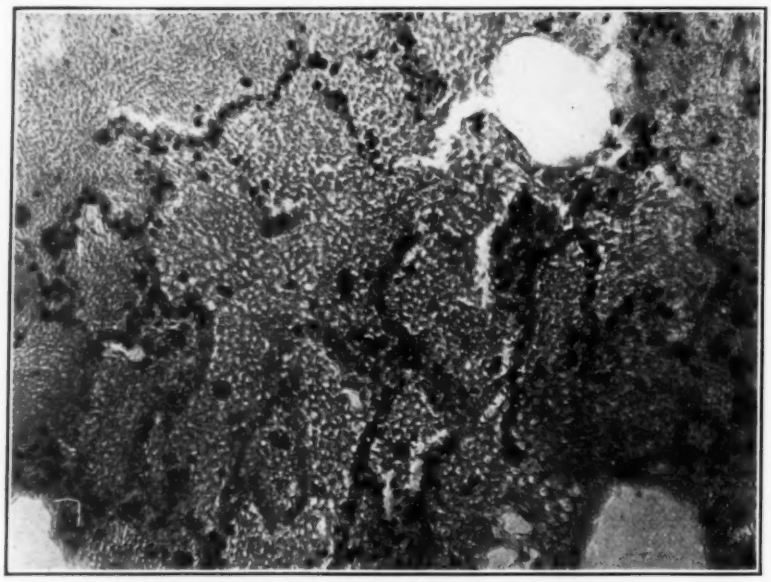

Fig. 3.—Congestion—air sacs filled with free red blood corpuscles.

taneously from the incision. The cut surfaces present a somewhat irregular appearance with patches of slightly emphysematous alveoli alternating with larger areas of edematous hemorrhagic tissue.

Microscopical.—In cases free from previously existing pulmonary lesions the alveoli are moderately dilated and the walls are outlined by deeply in-

Fig. 4.—Congested alveolar walls surrounding areas of fluid containing a few polymorphs.

Fig. 5.—(Low power.) Alveoli filled with mixed cellular exudate. The darker areas are masses of fibrin stained with phosphotungstic acid hematoxylin.

Fig. 6.—Infiltrated interstitial tissues with air sacs containing masses of pus cells.

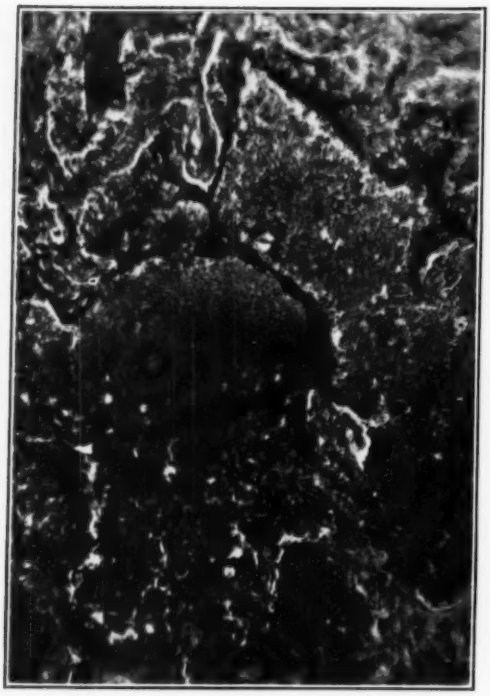

Fig. 7.—Alveoli outlined by fibrin strands. Exudate largely composed of red blood globules and blood pigment.

Fig. 8.—Section of portion of bronchus packed with cellular exudate. Walls are infiltrated with mononuclears and red blood cells.

jected arterioles. The air spaces contain either a homogenous clear acellular exudate (Fig. 2) or are packed by free red blood elements, (Fig. 3) and the lining cells are usually greatly enlarged and distorted by large single or multiple aqueous globules in the protoplasm.

The bronchioles generally contain a granular exudate in which there are a few red blood globules and detached epithelial elements.

In some cases, groups of air sacs are dilated to the point of fracture of the thin walls, while other groups are normal in size, but contain an homogeneous, gelatinous-appearing exudate with a few polymorphonuclear leucocytes scattered through the substance. (Fig. 4). In this setting the bronchi are always dilated, often filled with free blood and the walls are surrounded by mononuclear leucocytes.

In other cases the walls of the alveoli and the whole lung substance appear to be a mass of blood pigment, granular exudate, congested vessels, and newly formed fibrin.

Another type of lung condition frequently found is manifest by a few elevated patchy consolidations beneath the cyanotic edematous surface. Usually also there is a thin surface deposit of fibrin, particularly between the lobes. The substance of the lung is filled with dark purplish blood, and beginning elevated consolidations, firm and resistant to touch, are distributed in the central portions about the main bronchi near the hilum of the organ, from which point they diminish in size and number toward the apex and base. The bronchi contain a thin blood-tinged exudate, which in some cases is slightly purulent; the lumina is in many instances distinctly narrowed by the swelling of the mucous membranes. Blood capillaries of all tissues show most intense congestion, and the larger vessels are often filled with clots.

Microscopical.—Through these consolidated areas skeins of fibrin are strewn and lymphocytes are everywhere abundant. (Fig. 5.) In small localities there are groups of alveoli entirely filled with polymorphonuclear leucocytes; (Fig. 6) these alternate with others, which contain a granular exudate filled with red cells. (Fig. 7.) Catarrhal cells are numerous in all sections.

In some areas the bronchi are packed with pus cells, red blood globules and detached epithelial cells (Fig. 8). In lungs, the seat of bronchiectasis, the larger vessels are engorged with blood, the alveoli are thickened and heavily outlined by red blood corpuscles, and around the larger bronchi dense consolidation with acute inflammatory cells is present. In the lower lobes large areas of alveoli are filled with polymorphs.

When tuberculosis in any form is present, and where fibrous tissues are greatly increased, the tissues are especially rich in deeply congested vessels surrounded by small mononuclear cells. Highly pigmented catarrhal cells are very numerous.

THE HEART AND GREAT VESSELS

The majority of hearts in this series is the seat of a chronic interstitial myocarditis with tortuous, congested, atheromatous coronaries and nodular incompetent valve cusps.

In some cases all cavities are filled with large, red clots which sometimes

extend upward into the carotid branches and are whipped into the cordæ tendinæ.

Usually the right heart and whole pulmonary venous system is dilated and choked with fluid blood; and the auricular appendage often contains an enormous clot which strings along through the dilated ring of the tricuspid valve into the right ventricle where occasionally the cordæ tendinæ are very fragile and have ruptured under the increased strain.

In a few cases the muscle substance shows only a moderate cloudy swelling of the fibers, but in the greater number the tissue is very pale, softened and flabby.

When the valve cusps are free from atheromatous changes, they are apt to exhibit multiple minute hemorrhages.

TABLE I

INDICATING PRINCIPAL LESIONS AND PSYCHOSIS

NO.	AGE	SEX	ORIGINAL LESION	PSYCHOSIS
1	58	M	General Arteriosclerosis	Catatonic Dementia Precox
2	56	F	General Arteriosclerosis	Arteriosclerotic Dementia
3	40	M	Chronic Ulcerative Pulmonary TB	Paranoid Dementia Precox
4	50	M	General Syphilis	Cerebral Syphilis
5	72	M	Cerebral Arteriosclerosis	Manic Depressive Insanity
6	38	F	Massive Ascites	Dementia Precox
7	74	M	Pulmonary Abscess	Senile Deterioration
8	29	M	General Syphilis	Dementia Precox
9	57	M	General Arteriosclerosis	Dementia Precox
10	43	M	Chronic Ulcerative Pulmonary TB	Dementia Precox
11	45	M	Chronic Endo and Myocarditis	Undiagnosed
12	60	M	Osteomalacia	Senile Deterioration
13	47	M	Ancient Hemiplegia	Organic Brain Disease
14	50	M	Thyroid Hyperplasia	Dementia Precox
15	54	M	Chronic Ulcerative Pulmonary TB	Epileptic Psychosis
16	Aged	M	Fibrous Pleurisy	Epileptic Psychosis
17	59	F	Cerebral Arteriosclerosis	Arteriosclerotic Dementia
18	30	F	None	Dementia Precox
19	35	M	Early Miliary Pulmonary TB	Dementia Precox
20	24	M	Miliary TB (Pulmonary)	Dementia Precox
21	50	M	Diffuse Productive Nephritis	Arteriosclerotic Dementia
22	49	M	Chronic Myocarditis	Dementia Precox
23	43	M	Gangrene of Lung	Undiagnosed
24	64	M	General Arteriosclerosis	Undiagnosed
25	24	M	Chronic Pleuritis	Dementia Precox
26	49	M	Productive Nephritis	Dementia Precox
27	45	M	General Paresis	General Paresis
28	35	M	General Syphilis	Depression
29	38	M	Chronic Parenchymatous Nephritis	Dementia Precox
30	53	M	Generalized Miliary TB	Dementia Precox
31	44	M	Chronic Myocarditis	Dementia Precox
32	32	M	Bilateral Pyothorax	Dementia Precox
33	29	F	Chronic Ulcerative Pulmonary TB	Dementia Precox
34	78	M	Cerebral Arteriosclerosis	Senile Deterioration
35	74	M	Cerebral Arteriosclerosis	Senile Deterioration
36	70	M	Cerebral Arteriosclerosis	Arteriosclerotic Dementia
37	68	M	General Arteriosclerosis	Senile Deterioration
38	45	M	Chronic Ulcerative Pulmonary TB	Dementia Precox
39	48	M	Productive Nephritis	Dementia Precox
40	21	F	Miliary Pulmonary TB	Imbecility
41	7½ Mo.	M	None	
42	38	M	Internal Hydrocephalus	Imbecility

Microscopic.—In the most acute cases (i. e., short typical course with early termination) the capillaries are round, dilated and filled with red blood glob-

ules. In many zones the thin-walled structures have ruptured, allowing blood to diffuse into and between the muscle fibers which may be pigmented and widely separated.

In a few cases the heart muscle fibers are separated by long strings of corpuscles in rouleaux formation, the capillaries being intensely injected, the coronary arterioles packed with cells, and an occasional red thrombus present in the larger coronary branches.

Where the muscle cells have undergone extreme compensatory hypertrophy in chronic myositis there are separation, fragmentation, and albuminous degeneration of the fibers. In this extreme type the vascular changes are much less in evidence.

In other specimens focal separations of muscle occur, the spaces being filled with red blood corpuscles and hemoglobin pigments; in these there is much congestion and acute change in the cardiac capillaries.

Occasionally only clouding of the muscle fibers is noted. Acute cloudy changes in the cells, indistinct nuclei, increase in sheath elements and a moderate thickening of the vessel walls characterize the picture. In some cases of influenza in which there has been a previous myocarditis the only change present microscopically is that of chronicity, the acute process having apparently left no traces.

LIVER

In this series twenty-seven cases presented acute congestion with beginning fatty changes in the liver substance. In general, the organ was slightly enlarged, softened and edematous with yellowish patches on the surfaces. Cut surfaces were pale, yellowish and patchy yellow, with blood clots in the larger veins.

A few cases presented a deeply hemorrhagic substance containing much residual blood and in general cyanotic in appearance. Four cases showed a marked acute passive congestion and in three cases of portal cirrhosis, gross acute changes could not be demonstrated.

Microscopic.—The portal veins are notably dilated, and sometimes are distended almost to the point of rupture, the intralobular veins are packed with corpuscles, and the surrounding hepatic cells which are separated by clumps or masses of red blood corpuscles are undergoing acute fatty alteration.

The bile ducts are dilated, the lining cells in states of degeneration, and there is diffuse granular pigment scattered through the tissues, especially in cases where the chronic cellular elements are notably increased in the portal canals. The bile pigments are markedly increased in the organs, presenting a thickened capsule, retrograde hepatic cells, proliferating bile capillaries and new formation connective tissue.

Occasionally the hepatic cells are widely separated and distorted, showing extreme fatty alteration, many being mere rims of protoplasm, the center of which is filled with large globules. The shrunken cells are separated by a finely granular deposit which contains many red blood cells.

Central lobular acute cellular changes are frequent and often the cells are swollen edematous and indistinct in nuclear outlines.

Wherever fibrous tissue has previously formed as in portal cirrhosis, the newly formed blood capillaries stand out prominently from congestion. The thickened fibrous capsules are well studded with congested capillaries and the tissues contain many free blood cells. In these situations where islands of liver tissue have long been exposed to pressure from the strands of thickened connective tissue, the cells show localized areas of acute destruction; the spaces left from the complete necroses are occupied by clumps of red blood corpuscles from the surrounding ruptured vessels.

SPLEEN

The most frequent changes noted in these spleens are moderate enlargement, cyanotic surfaces often with dark mottlings, and rather firm on palpation; and a bright, softened, easily fractured substance.

Sections disclose a soft, swollen pasty pulp loaded with semifluid blood. Twenty-eight cases are of this variety.

In four instances hemorrhagic perisplenitis with pigmentation of splenic substances occurred. In only one case is the spleen pale in color, and in this the softening of the pulp is well marked.

In seven cases the spleen was originally of the chronic interstitial type, but presents acute congestion, a creamy pink pulp and a softening of the lymphoid structures.

Microscopic.—In the acutely congested types there is an excessive amount of blood pigment distributed between the cells of the pulp, the sinuses engorged with red blood cells or granular debris, the endothelial cells in stages of proliferation, and the arterioles exceptionally large and filled with blood. Instead of the diffuse picture the splenic pulp may be dotted by irregular patches of dense hemorrhage, with a striking congestion of the arterioles of the malpighian bodies.

Hyaline degeneration of the vessels is relatively frequent and the lymph nodules may be surrounded by hemorrhagic zones.

When there is a chronic splenitis with an increase in trabeculæ, the blood vessels of this tissue stand out very prominently from congestion, and beneath the thickened capsules, zones, filled with minute hemorrhages are frequent. Many endothelial phagocytes bearing blood pigments are scattered in these regions.

The larger vessels in general present a pale, homogeneous-appearing media and an occasional hyaline degeneration or thrombotic formation.

KIDNEYS

In nearly all the cases, regardless of the original type of lesion present in the kidneys, an acute change could easily be demonstrated. (Table II.) The organs are slightly tense from swelling, and general congestion; the stellate veins deeply injected; the cut surfaces edematous, granular, everted, often fatty, and deeply hemorrhagic; the pyramids and papillæ enlarged and congested, and the larger vessels often filled with dark red blood clots.

Microscopic.—Microscopically, they may be arranged into two groups, viz.,—

Group 1, where the changes are acute only, and Group 2 with two subdivisions, where acute processes are superimposed upon chronic kidney disease.

In Group 1 the interstitial tissues, including the capsule, contain minute hemorrhages, where the small capillaries have ruptured and thrown their

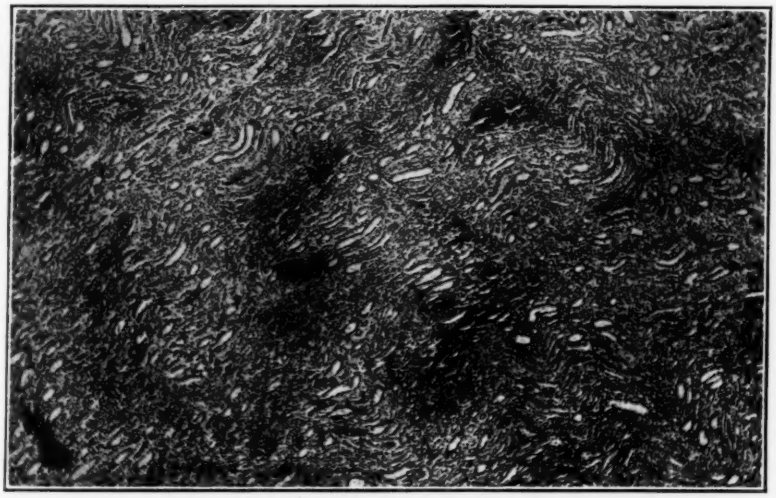

Fig. 9.—(Low power.) Dark areas indicate intratubular and intertubular hemorrhages.

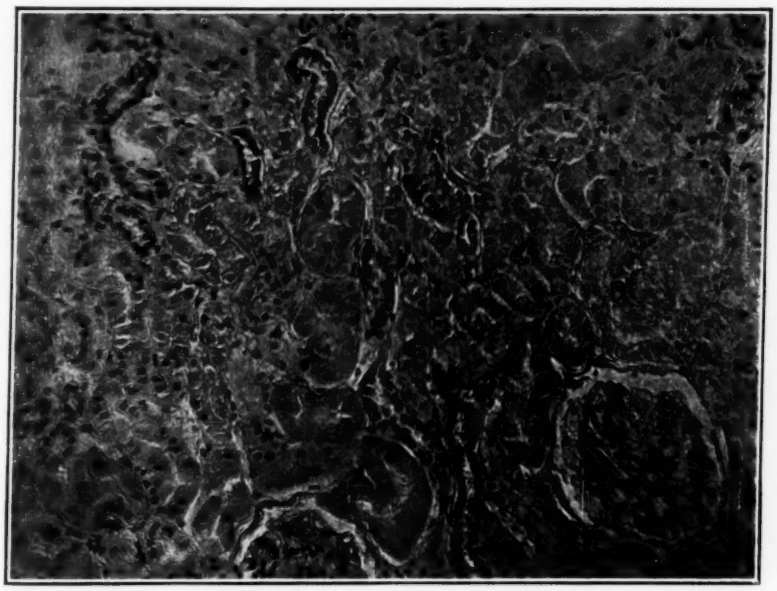

Fig. 10.—Simple cloudy swelling of the parenchymatous cells.

contents into the surrounding tissues. Hemorrhage is usually most profuse immediately beneath the capsule, where masses of free blood corpuscles are distributed over wide areas. Intertubular hemorrhages are present in all fields, but are more abundant through the collecting tubule regions where the thin-walled, congested capillaries have partially disintegrated. (Fig. 9.)

Some glomeruli are very hemorrhagic, the spaces filled with finely granu-

TABLE II

ACUTE CHANGES IN THE KIDNEYS

CASE NO.	EDEMA AND CONGESTION	ACUTE PARENCHYMATOUS CELL CHANGES	HEMORRHAGES
Group I. Without Chronic Kidney Lesions.			
3	Slight	Slight	None
8	Intense	Advanced	Numerous intratubular hemorrhages and hemorrhagic tufts
10	Marked	Moderate	Diffusely hemorrhagic substance
11	Intense	Moderate	Hemorrhages into glomeruli and into interstitial tissues
14	Marked	Advanced	Few hemorrhages through collecting tubules
16	Moderate	Moderate	Minute hemorrhages through interstitial tissues
17	Marked	Moderate	Numerous intra and intertubular hemorrhages
18	Slight	Slight	None
19	Slight	Advanced	Intratubular hemorrhages
20	Marked	Marked	Intratubular hemorrhages and hemorrhagic glomeruli
21	Marked	Advanced	Intratubular hemorrhages and hemorrhagic glomeruli
22	Marked	Slight	None
25	Marked	Advanced	Very numerous intertubular hemorrhages
27	Marked	Slight	Few hemorrhagic glomeruli
29	Slight	Slight	Few ruptured capillaries
30	Moderate	Slight	None
31	Marked	Moderate	Hemorrhages into tufts
32	Marked	Moderate	Hemorrhagic glomerulitis and intertubular hemorrhages
40	Marked	Marked	Hemorrhagic glomerulitis
41	Marked	Marked	Patchy interstitial hemorrhages
42	Marked	Marked	Intertubular hemorrhages
Group II (a) With Chronic Kidney Lesions (Diffuse Productive Nephritis).			
1	Marked	Advanced	Hemorrhagic interstitial tissues
4	Marked	Advanced	Enormous number of small hemorrhages in all structures
6	Marked	Advanced	Very numerous intertubular hemorrhages
9	None	Moderate	None
12	Moderate	Advanced	Numerous interstitial hemorrhages
13	Marked	Marked	Diffuse hemorrhages between tubules and into glomeruli
15	Marked	Slight	Hemorrhagic glomeruli
23	Marked	Advanced	Hemorrhages into tufts and into interstitial tissues
24	Moderate	Moderate	Few hemorrhages into glomeruli
26	Marked	Advanced	Numerous subcapsular hemorrhages
28	Marked	Advanced	Diffusely hemorrhagic substance
33	Moderate	Moderate	Glomeruli moderately hemorrhagic
34	Marked	Marked	Few small intertubular hemorrhages
35	Marked	Marked	All portions deeply hemorrhagic
36	Marked	Marked	All portions deeply hemorrhagic
38	Marked	Marked	Hemorrhagic glomeruli
39	Marked	Marked	Small inter and intratubular hemorrhages
Group II (b) With Chronic Kidney Lesions (Patchy Productive Nephritis).			
2	Moderate	Early stages	Diffuse hemorrhagic glomerulitis
5	Marked	Moderate	All glomeruli hemorrhagic—patchy intratubular hemorrhages
7	Slight	Slight	None
37	Marked	Marked	Numerous intertubular hemorrhages

lar exudate and the tuft cells swollen, while in others the notable features are dilatation of the capsules of Bowman with profuse hemorrhages in the periglomerular spaces.

The tubular cells are swollen, irregular, fatty, fragmented and displaced from the basement membrane (Fig. 10), with many of the collecting tubules containing red blood corpuscles, hyaline casts, granular parenchymatous cells and a few polymorphonuclear leucocytes.

Where inter- and intratubular hemorrhages are most numerous, the parenchymatous cells are loosened and necrotic.

In the case of pregnancy the parenchymatous cells showed acute widespread destruction with pale, necrotic, disintegrating substance.

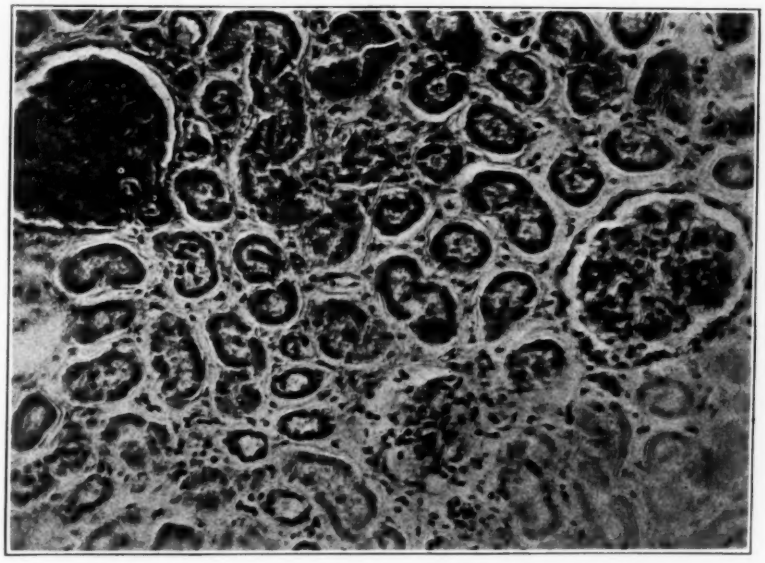

Fig. 11.—Productive nephritis with advanced acute degeneration of the flattened tubular cells.

Microscopic Group 2 (a):

The following is the microscopic picture of influenza generally seen in a kidney which is the seat of a diffuse productive nephritis.

In this group of cases the capsule which is already thickened by proliferated fibrous tissue contains large engorged dilated vessels, many of which are thrombotic or present hyaline changes in their walls.

Underneath the fibrous hemorrhagic capsule the vessels are thin-walled, dilated, and packed with red blood corpuscles. In patches there is fibrous obliteration of subcapsular structures in the midst of which hyaline degeneration of cell masses is a frequent feature.

In some cases the acute changes are limited to the glomeruli, in which the tuft vessels are enlarged and packed with corpuscles. Again the glomeruli may be knotted, with the tuft cells undergoing fatty degeneration, and the spaces filled with a granular hemorrhagic exudate.

Fibrous tissue has abundantly proliferated, destroying the parenchymat-

TABLE III

ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENINGES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
1	Diffuse gliosis	Considerable	Edematous	Sulci filled with fluid	Edematous	Congested	Edematous
2	Arteriosclerotic softenings	Increased	Rusting of pia mater. Deeply congested	Deeply congested	Deep congestion, dilated spaces and acute softening	Glistening surface exudate	Deep congestion and punctate hemorrhages
3	None	Marked excess	Vessels and sinuses engorged	Surfaces oozing blood — marked edema	Softened, boggy and hemorrhagic	Dilated—greatly congested	Great congestion of the substance of pons and cerebellum. Basal ganglia soft and hemorrhagic
4	Sclerotic atrophy of right hemisphere	Increased	Marked congestion	Edematous, hemorrhagic	Edematous	Not remarkable	Not remarkable
5	General senile atrophy	Increased	Vessels congested	Edematous	Diffuse blood pigment in all areas	Markedly congested and dilated	Numerous punctate hemorrhages through the internal capsule and gray ganglia
6	Patchy gliosis	Diminished	Slight congestion	Boggy	Moderate edema	Moderate congestion	Edematous
7	Senile atrophy	Marked excess	Basilar congestion. Pial clouding	Multiple softenings and hemorrhagic points	Edematous	Distended vessels	Very edematous—pons and medulla hemorrhagic
8	None	Slight excess	Marked congestion	Pale and moderately soft	Acute softenings	Markedly congested	Basal ganglia notably edematous
9	Cerebral arteriosclerosis	Marked excess	Strips easily. Deeply congested and milky	Subarachnoid diffusions of blood	Edematous	Dilated—ependyma congested	Diffuse congestion
10	Focal cortical atrophies	Excess	Slight congestion	Congested and presents minute hemorrhages	Edematous, few petechial hemorrhages	Diffuse red discolorations of ependyma	Edema and congestion of all structures
11	None	Normal amount	Sinuses filled with clots, intense congestion	Very edematous. Subpial hemorrhages	Dilated lymph spaces. Few hemorrhages	Slightly congested	Basal ganglia show few punctate hemorrhages
12	Cerebral arteriosclerosis	Normal	Congested. Large hemorrhages	Notably edematous. Few acute softenings	Few acute softenings	Ventricles dilated. Ependyma congested	Numerous prominent punctate hemorrhages through the basal ganglia
13	Sclerotic atrophy of left hemisphere	Marked excess	Extremely congested	Very hemorrhagic and edematous	Presents numerous softenings	Ventricles greatly dilated. Ependyma is congested and granular	Central softening in optic thalamus
14	None	Excess	Intensely congested	Superficially anemic and edematous	Punctate hemorrhages	Ependyma reddened and glistening	Softenings in internal capsules and lenticular nuclei. Splenium studded with punctate hemorrhages

TABLE III—Continued

ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENINGES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
15	Cortical atrophy	Marked excess	Slight congestion	Thin, edematous. Very few hemorrhages	Numerous punctate hemorrhages through the occipital portion	Slight congestion of the ependyma	Pons medulla and cord show acute diffuse softening
16	Sclerotic atrophy of the left temporal lobe	Increased	Diffuse intense congestion	General edema	Very hemorrhagic	Ventricles moderately dilated. Ependyma congested, large vessels	No remarkable changes
17	Cerebral arteriosclerosis	Normal	Milky congested pia	Extensive hemorrhages and softenings	Softenings and hemorrhages	Normal in size	Multiple softenings in the basal ganglia
18	None	Normal	Deeply congested	Superficial softening with some petechial hemorrhages	Marked edema	Ependyma dull, cloudy granular	Small area of softening in right thalamus. Small hemorrhages in internal capsules. General edema
19	None	Moderate excess	Meninges strip easily Deeply congested	Pale, boggy, lower layers hemorrhagic, anemic surface	Not remarkable	All ependyma pale, lusterless	Surface congestion. Basal ganglia spotted with minute hemorrhages
20	None	Normal	Congested and edematous	Thin, pale and boggy. Few punctate hemorrhages	Filled with free red blood pigment	Dark red congested ependyma	Minute hemorrhages are through the basal ganglia, the internal capsule, the pons, the medulla and spinal cord
21	Cerebral arteriosclerosis with multiple softenings	Marked success	Intensely congested; Diffusely opaque	Hemorrhagic	Hemorrhagic. Diffuse blood pigments	Ventricles dilated. Deeply congested ependyma	Basal ganglia and internal capsules show many arteriosclerotic degenerations, small free hemorrhages are numerous
22	None	Normal	Large veins with coagulated blood—membrane infiltrated with hemorrhages	Considerable free blood over the surface	Diffuse pink in color	Ependyma pink lusterless. Choroid plexus deeply congested	All structures soft, edematous and present punctate hemorrhages
23	None	Normal	Congestion of both dura and pia	Reddened from blood pigments	Edematous	All surfaces pale, lusterless and ependyma presents small hemorrhages	Basal ganglia studded by minute hemorrhages and dilated vessels
24	Cerebral arteriosclerosis	Normal	Marked congestion and subpial hemorrhages	Hemorrhagic	Edematous	Ependyma covered by bloody exudate	Minute hemorrhages through the internal capsules. Thalamus and corpus striatum are markedly edematous

TABLE III—Continued

ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENINGES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
25	None	Normal	Deep congestion of entire surface and widespread blood diffusion	Minute hemorrhages and superficial acute softening	Pink from diffused blood	Ependyma presents deep red dull surface	All capillaries of cerebellum and pons exceptionally prominent. Punctate hemorrhages through the basal ganglia, internal capsule and corpus callosum
26	None	Marked increase	All membranes intensely congested	Deeply congested	Deeply congested	Not remarkable	Vessels of cerebellum and pons greatly dilated. Minute hemorrhages in basal ganglia and in corpus callosum
27	Paretic neurosyphilis	Marked excess	Deep congestion over all surfaces	Cloudy, edematous and hemorrhagic	Moderate blood diffusion	Ventricles present diffusely congested surface	The corpus callosum and all basal ganglia are mottled with minute hemorrhages and edematous lymph spaces
28	Syphilitic meningitis arteriosclerotic softening of left occipital lobe	Marked excess	Intense congestion of entire surface	Presents numerous acute softenings and congestion	Edematous and congested	All ventricles dilated	Basal ganglia are atrophied and hemorrhagic, acute softenings through the internal capsules
29	Chronic leptomeningitis	Moderate excess	Diffuse congestion of all membranes	Cloudy and granular	Edematous	Ventricles greatly dilated. Ependyma congested	The basal ganglia and internal capsules are strikingly edematous and contain many petechial hemorrhages
30	Generalized atrophy	Marked excess	Diffusely congested	Edematous	Softening about ventricles and presents many ruptured vessels	Dilated ventricles Congested	Basal ganglia evince edema and congestion
31	None	Slight excess	Edematous and congested	Contains numerous minute hemorrhages	Hemorrhagic	Congestion of ependyma	The basal ganglia particularly the lenticular nucleus is dotted with minute hemorrhages. Pons deeply congested
32	None	Slight excess	Marked congestion	Minute capillaries, prominent	Generalized congestion and widened lymph spaces	Ependyma is pale, dull and boggy	Substances of pons and cerebellum are moderately hemorrhagic. Softened lenticular nuclei all ganglia are hemorrhagic. Corpus callosum is hemorrhagic
33	None	Moderate excess	Moderate congestion and blood diffusion	Intense congestion	Edematous and filled with punctate hemorrhages	Ependyma smooth and dull, pink in color from blood pigments	Basal ganglia filled with punctate hemorrhages substances of pons, medulla and cerebellum are congested

TABLE III—Continued

ORIGINAL AND ACUTE BRAIN LESIONS

CASE NO.	ORGANIC BRAIN LESIONS	SPINAL FLUID	MENINGES	CEREBRAL CORTEX	CENTRUM SEMIOVALE	VENTRICLES (EPENDYMA)	BRAIN STEM
34	Cerebral arteriosclerosis. (L. V. T.)	Notable increase	Entire surface congestion	Deeply congested	Deeply congested	Dilated ventricles. Ependyma dark red. Brown blood pigments	Basal ganglia are hemorrhagic
35	Cerebral arteriosclerosis (L. V. T.) Cranial nerve atrophies	Moderately increased	Diffusely congested	Extremely thin and pale	Edematous.	Ependyma diffusely red and loosened	All ganglia and internal capsules are discolored and softened
36	Cerebral arteriosclerosis (L.V.T.) with sclerotic atrophies	Enormously increased	Engorgement of vessels	Few acute softenings	Hemorrhagic	Ependyma granular and congested ventricles widely dilated	Basal ganglia and corpus callosum with the capsules are markedly congested and present punctate hemorrhages
37	Senile atrophy	Marked increase	Marked congestion and hemorrhages	Thin, edematous and hemorrhagic	Edematous	Ependyma congested	Basal ganglia show numerous petechial hemorrhages with acute softenings
38	None	Moderate increase	Intensely congested	Markedly hemorrhagic	Filled with punctate hemorrhages	Choroid plexus swollen. Ependyma covered by hemorrhagic exudate	Substance of pons, medulla and and cord is filled with petechial hemorrhages. Basal ganglia edematous and hemorrhagic
39	None	Slightly increased	Diffuse congestion	Edematous acute softenings, hemorrhagic	Thickly studded with minute hemorrhages	Ependymal capillaries distended, membrane dull, pink in color	Substance of pons and medulla are filled with minute hemorrhages. Basal ganglia, corpus callosum and internal capsule are also hemorrhagic
40	Microcephalia	Normal	Vessels engorged	Edematous and pale	Hemorrhagic	Large vessels engorged	Basal ganglia acutely softened and very hemorrhagic
41	Fetus	Normal	Intensely congested. Much free blood	Hemorrhagic and soft	Softened, disintegrate	Congested	Many vessels ruptured. Substance disintegrated
42	Hydrocephalus internal	Enormous increase	Intense congestion	Petechial hemorrhages	Vessels markedly distended	Ependyma congested, ventricles enormously enlarged	Basal ganglia flattened and distorted from pressure, but very hemorrhagic and edematous

ous structures over large areas in which hemorrhages from small ruptured vessels are seen in large numbers.

In most instances there is an acute albuminous degeneration of the living tubular cells, which are pale, bear indistinct nuclei and sometimes hyaline changes, and the lumina of the tubules contain a finely granular exudate.

The collecting tubule regions are characterized by extra- and intratubular hemorrhages, many of which cover large areas with diffusion of red blood cells; and by the acute changes in the parenchymatous cells which are greatly swollen, nearly filling the tubules, and are often indistinct without visible nuclei.

Microscopical of Group 2 (b): In this group of arteriosclerotic kidneys multiple small interstitial and intratubular hemorrhages are marked in the patchy sclerotic zones where congested areas seem to alternate with strips bearing normal appearing vessels.

There is a general hemorrhagic glomerulitis with much blood pigment and wide edematous spaces. The convoluted tubules present flattened, granular, cloudy cells, sometimes in stages of disintegration. (Fig. 11.) Some of the collecting tubules contain masses of red blood corpuscles, and hyaline casts are especially numerous. A few polymorphonuclear leucocytes are occasionally seen in the collecting tubule regions.

GLANDS OF INTERNAL SECRETIONS

Thyroid.—Nearly all of the adult thyroids show atrophic features and a chronic interstitial thyroiditis. Congestion with abundant deposits of blood pigment are the constant acute changes.

Adrenals.—These glands are usually swollen and dark colored—actual gross hemorrhages are not seen.

Microscopically there is a patchy cloudy swelling of the parenchymatous cells of the cortex, a few focal necroses, and a number of interstitial hemorrhages. The medullary portion is congested and contains many lymphocytes.

Pituitary Glands.—Show no remarkable acute features.

Pancreas.—This organ is most often swollen, congested and softened, but is occasionally pale and firm.

Microscopically all the larger vessels are engorged with blood, the capillaries prominent with congestion, and a few interstitial hemorrhages are noted. The cells of the alveolar glands and of the pancreatic ducts are in some cases irregular, shrunken, and heavily stained, while in other instances they are swollen cloudy and indistinct.

The islands are usually small, pale, and composed of a few widely separated cells. The pancreatic ducts are filled with granular debris and their walls are strikingly outlined by intensely congested capillaries.

CENTRAL NERVOUS SYSTEM

Table III gives the original and acute gross brain lesions.

Meninges.—The cerebrospinal fluid is usually in moderate excess and the dura presents deeply congested vessels and engorged sinuses.

In the pia the intricate networks of capillaries are prominent over all sur-

faces of the hemispheres. The pia of the cerebrum, because of its edema, strips easily, is milky in color and is frequently opaque along the vessel sheaths.

Blood diffusion through the arachnoid meshes is seen in many specimens in which the surfaces ooze blood, where veins are dilated to enormous size, and in which free blood pigments are abundant in the depths of the sulci. In some cases the pial congestion is more pronounced over the sylvian areas, where the veins are engorged to about three times the usual size, contain solid casts of blood, and originate large hemorrhages, which extend beneath the membrane to the surrounding tissues; in others, the vessels over the frontal and parietal lobes are more prominent and contain large quantities of blood, some of which has diffused out through the arachnoid spaces; and in still other cases the bases of the frontal and temporal lobes are most intensively congested.

The gaping sulci of the senile atrophic cases are covered by cloudy membranes, which contain many bright-red capillaries, and the depths of the fissures contain much free blood, which often diffuses inward as far as the basal ganglia. The cerebellar pia is often edematous and milky-white in color, but seldom exhibits more than a slight injection of the capillaries.

Cerebral Cortex.—This structure usually presents a slight brownish tinge. These red pigments are deposited through the cortex and have infiltrated the white substance, having extended from the congested vessels of the numerous sulci, where in the depths this change is marked. The cortex and white matter of the convolutions are filled with edematous spaces, multiple acute softenings, and hemorrhagic points.

In some instances the parietal operculum is particularly hemorrhagic in its cortical zones. The acute changes are often very prominent, particularly softenings in both the cortex and white substance of the Islands of Reil.

BRAIN STEM STRUCTURES

The basal ganglia are soft, boggy, filled with petechial hemorrhages, and present a diffuse pink color. In the thalami and in the caudate nuclei the hemorrhages are somewhat larger and these structures often exhibit around the larger central vessels acute softenings which extend through to the floor of the ventricle.

The internal capsule regions contain many small hemorrhages, are darkened in color, and are extremely edematous.

The ependyma of the occipital extensions of the lateral ventricles is characterized by a bloody, slimy surface deposit, and dilated vessels. In general the ependyma of the lateral ventricles presents considerable blood diffusion, and a discolored, cloudy surface deposit composed largely of blood, cerebrospinal fluid, and loosened membranes. The large vessels of the sub-ependymal regions are always engorged with blood.

The surfaces as well as the substance of the cerebellum and pons are deeply congested and contain punctate hemorrhages, about which minute areas of necrosis are visible.

MICROSCOPICAL CHARACTERISTICS OF THE BRAIN SECTIONS

Frontal Lobes.—The pia mater contains dilated thin walled, congested vessels; and large numbers of red corpuscles have poured into the enlarged fluid filled arachnoid spaces. When the membranes extend into the sulci there are deposited many red blood cells, lymphocytes, and a few plasma cells.

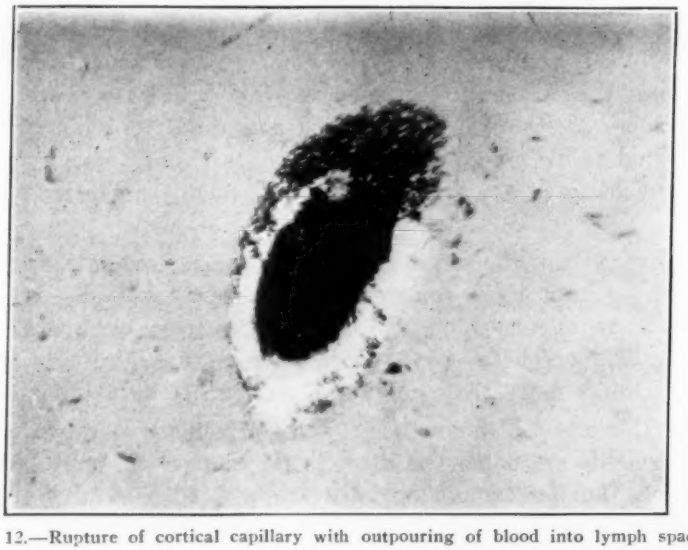

Fig. 12.—Rupture of cortical capillary with outpouring of blood into lymph space.

Fig. 13.—Hemorrhages into sclerotic patch in a section of frontal cortex.

The blood vessels of the cortex even to the smallest capillaries are filled with red blood corpuscles or thrombi and are surrounded by widened perivascular lymph channels many of which are filled with hemorrhages. (Fig. 12.)

Extravasations of blood into the tissues are present through the cortex and in the white matter. The central portions of these hemorrhages are necrotic

and loaded with hematogenous pigment and the periphery also exhibits a diffuse distribution of hemosiderin.

In some of the cases of arteriosclerotic brain disease patches of focal sclerosis are conspicuously prominent from hemorrhages occasioned by the rupture of the weakened walls of the new formation vessels. (Fig. 13.)

The superficial layers of nerve cells are swollen, react weakly to stains and many present an irregular areolar appearance. The deeper cell layers are imbedded in diffusely edematous surroundings; some cells are vacuolated and present varicose dendrites, a few have undergone hyaline degeneration and are surrounded by wandering cells, while others are in stages of advanced chromatolysis.

Through the white matter there is an unusual number of mononuclear leucocytes, the general picture being one of acute edema with beginning encephalitis.

Precentral Gyrus.—The Betz cells in general are rather pale, with an in-

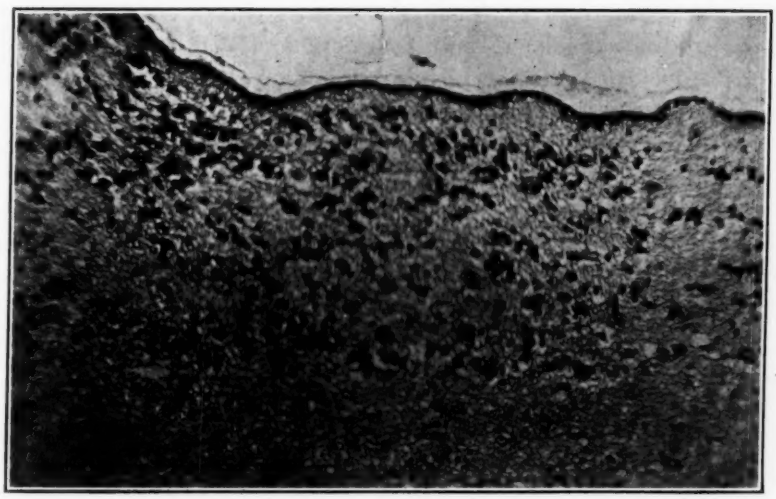

Fig. 14.—Infiltration of lymphocytes in the subependymal structures of the thalamus.

crease in pigment in the axone "hills". In some places the cells show fatty changes, hyaline alterations, and fragmentation of the chromatin granules, while in others the nuclei are heavily pigmented eccentrically situated, and in some cells showing only the nucleolus.

The smaller cortical parenchymatous cells are cloudy, nuclei indistinct and many of the smaller ones are more than half replaced by yellow globular pigmentation. In some areas these cells present fragmented chromatin marginally arranged, and showing strong basic staining properties. A few cells are fatty and elongated while others are contracted and heavily stained. The vascular changes are similar to those mentioned under the frontal lobes.

Hippocampus.—Punctate hemorrhages are noted in these structures, but most of the nerve cells appear exhausted. In addition to the acute changes the arteriosclerotic cases show pigmentation of the cells, some of which are completely replaced by yellow pigment, others exhibiting only central pigmen-

tation and still others with only a thin margin of original cell structure remaining. Mononuclear phagocytes are quite numerous.

Cerebellum.—The pia in the depths of the sulci shows a moderate lymphocyte infiltration and an engorgement of all vessels.

The molecular layer contains many lymphocytes and presents an occasional hemorrhage into the substance.

The purkinjin cells show exhaustion, fragmentation with irregular distribution of chromatin, some cloudy swelling, and numerical reduction especially along the lateral positions on the leaflets where only the faint outlines of many cells remain. In some specimens the purkinjin cells are slightly enlarged, and heavily stained, showing diffusion of chromatin through the cytoplasm.

Thalamus.—The overlying ependyma is usually intact, rather cloudy, and reacts weakly to stains. The subependymal structures are diffusely infiltrated with lymphocytes. (Fig. 14.)

In the brains showing advanced organic vascular changes the ependymal cells are distintegrating and the subependymal tissues present dilated perivascular lymph spaces and an occasional minute hemorrhage, about which mononuclear wandering phagocytes collect in moderate numbers.

The substance of the thalamus is edematous and all vessels are prominent from congestion. The walls of many of the larger vessels are in stages of early hyaline degeneration while others are thrombosed. A large proportion of the nerve cells appear swollen and heavily stained, but few are fragmented and in many of the older previously affected specimens, the nerve cells present the additional yellow globular pigment in the cytoplasm and an occasional pigmented nucleus.

Lenticular Nucleus.—There is a deep congestion of the substance, but very few hemorrhages, and no perivascular necroses are seen. The nerve cell alterations resemble those of the thalamus.

Choroid Plexus.—The cells of the villi evince acute hypertrophy. Numerous engorged vessels have ruptured pouring out red blood globules which are everywhere in evidence. Dilatation of the larger vessels and bright congestion of the minute capillaries are the striking features.

Medulla.—Many ruptured vessels are seen with surrounding zones of necrotic alterations, and accumulations of blood pigment are frequently noted near the nerve cells, which are sometimes granular, but usually are heavily stained a deep blue from the diffusion of chromatin. In many the cytoplasm has apparently burst pouring its substance out into surrounding spaces.

The capillaries of the motor regions are deeply congested and are surrounded by lymphocytes. The lymph spaces occasionally contain a few polymorphonuclear leucocytes.

Olives.—Most of the changes appear acute with intense congestion of the vessels, the walls of which are pale, weakened and ruptured producing small hemorrhages, and surrounded by lymphocytes filled with blood pigments, as are also those in the perivascular lymph spaces. Many other vessels with walls intact, but choked with blood are observed. The nerve cells have swollen nuclei, and a protoplasm which is reduced in amount and generally granular.

Pons.—The pons tissue shares in the general congestion and the nerve cells

of the nuclei are swollen, vacuolated and often fragmented. In many cells the chromatin presents a marginal arrangement in the cytoplasm or is occasionally massed in large pale staining lumps toward the center of the cell structure.

Spinal Cord.—The cord structures present an unusually intense congestion and numerous lymphocytes and plasma cells collect in the vicinity of the central canal. Some of the anterior horn cells appear fairly normal showing only a slight cloudiness, but in general the acute alterations in the nerve cells are notable. There is a tendency to clumping of the chromatin material, and in most cells there is a marked reduction in the number of nissl bodies.

CONCLUSIONS

1. Practically all tissues of the body presented acute changes, the result of the infection; these changes were congestion, edema, degeneration, and rupture of walls of blood vessels resulting in hemorrhages and focal necroses, and alterations in the parenchymatous cells varying in degree from simple albuminous degeneration to complete necrosis. When the organs were the seat of chronic processes having an abundance of new formation blood vessels there was a striking hemorrhagic picture produced from the rupture of these vessels, and the associated tissue reactions.

2. Mucous membranes in general exhibited one or the other of two changes, the congested vessels were plainly visible with bright capillary networks or the membranes were a diffuse beefy red color from rupture of vessels and general outpouring of red blood globules.

3. In 21 cases the kidneys were fairly free from chronic disease, but reacted strongly to the infection by acute parenchymatous cell alterations, marked general edema and universally by hemorrhages, which varied in number, size and location. In the 21 cases forming Group II of original productive nephritis, the acute changes were more diffuse and destructive, particularly the hemorrhages which were often remarkable in extent.

4. The distribution of the chronic organic brain lesions among the clinical divisions were as follows:

MENTAL DIAGNOSIS	NUMBER OF CASES	ORIGINAL LESIONS	ABSENT
Dementia Præcox	20	6	14
Arteriosclerotic Dementia	5	5	0
Senile Deterioration	5	5	0
Manic Depressive Insanity	2	2	0
Neurosyphilis	2	2	0
Undiagnosed	3	2	1
Epileptic Psychoses	2	2	0
Imbecility	2	2	0
Fetus	1	0	1
Total	42	26	16

Of the twenty cases clinically diagnosed dementia præcox, six showed original organic brain disease usually of the nature of a diffuse, or of a focal gliosis, while congestions, hemorrhages, and acute softenings were prominent through all structures regardless of the presence or absence of an original lesion.

In the brains from senile and arteriosclerotic patients presenting the usual vascular changes and lack of adequate nutrition, the acute process was

exceptionally destructive to vessel walls, and focal areas of softening were most abundant.

5. Although the above acute pathologic changes occurred in brain tissue taken from individuals with psychoses, and organic cerebral lesions, and perhaps somewhat more susceptible to further alteration, one might suppose and indeed it has been found to be the case, that these acute changes are present in varying degrees in brain tissue from those previously free from neurological and mental symptoms.

6. Among the influenzal and postinfluenzal psychoses, described by numerous writers, the acute hallucinatory disorders, depressions and dementia præcox were the most frequent. The intense meningeal and cortical edema and congestion, the acute processes in the parenchymatous cells, and the alterations in the vessel walls may account for the precipitation of many cases of acute hallucinatory disorder. In later stages of cerebral edema there has been evidence of a tendency to develop depressions, many of which are of the type indicated by the term Manic Depressive Insanity.

According to Menninger and others many cases of the dementia præcox group, and other major psychoses have passed through a febrile or postfebrile delirium and have shaded gradually into the more prolonged or permanent condition; a state which in those with a latent tendency may perhaps be determined, or aroused to activity by the cortical and subcortical necroses produced by small thromboses and ruptured vessels.

Neurasthenia with its characteristic fatigue without energy expend has been the most frequent postinfluenzal neurosis, and might also be considered in the above statement.

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THE ALKALI RESERVE OF BLOOD PLASMA DURING ACUTE ANAPHYLACTIC SHOCK*

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A DECREASE of the alkali reserve of blood plasma of animals during shock resulting from intravenous injection of toxic foreign proteins having been observed, a group of acute anaphylactic experiments were outlined in order to make further observations upon these changes in toxemic shock. The results observed follow.

TECHNIC

In these experiments rabbits, dogs, and guinea pigs were used for the production of anaphylactic shock. These animals were sensitized by preliminary injections of horse serum, or purified dried egg albumen. The influence of the preliminary or sensitizing doses of protein upon the carbon dioxide capacity of the plasma was studied and found negligible. The animals, after receiving the sensitizing doses of protein, were kept upon a well balanced diet for an interval sufficient for sensitization, at the termination of which the shock experiments were made. Preliminary observations of the alkali reserve of the blood plasma were made upon every series of animals used in the experiments for several days before the production of shock, to determine the normal daily variations of the animals. Following these preliminary tests the animals were bled immediately previous to, and at regular intervals following, the injection of the shock dose of foreign protein.

EXPERIMENTS UPON RABBITS

Eighteen rabbits were sensitized by the intravenous injection of 2 c.c., 5 c.c. and 8 c.c. of horse serum at four-day intervals. After the last sensitizing dose preliminary tests were begun to determine the daily variation of the alkali reserve in these animals. Marked daily variations in the carbon dioxide capacity occurred in the rabbits without apparent cause which made the proper interpretations of the findings in these animals questionable. The experiments were continued, however, with the following results:

Eighteen sensitized rabbits received 5 c.c. of horse serum intravenously as the toxic dose. The following table represents the average data obtained in these animals. As the findings of eighteen animals are included in the table, the results probably represent the changes which occur in the alkali reserve of rabbits during anaphylactic shock. (See Table I.)

The symptoms of acute anaphylactic shock in rabbits are not produced with any degree of uniformity and may only become manifest after several days. However, several animals included in the table showed acute symptoms. All

*From the Department of Pathology of Manhattan Eye, Ear and Throat Hospital, and College of Physicians and Surgeons (Columbia University).

TABLE I

Average percentage carbon dioxide capacity of 18 rabbits in anaphylactic shock
Animals received last sensitizing dose March 17, 1919

DATE			C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. OF PLASMA
April 8, 1919			56.7
April 9, 1919			45.9
April 10th			59.1
April 11th—	9	A.M. before shock	54.2
	9:30	A.M.—injection 5 c.c. horse serum	—
	11	A.M.	36.3
	3	P.M.	50.2
	6	P.M.	57.3
April 12th	9	A.M.	50.3

the rabbits became emaciated and several died in four to six days. The delayed effect of anaphylactic shock in rabbits and the normal variations of the alkali reserve of the blood in these animals, with no apparent cause, render them unsatisfactory for these experiments and they were abandoned. In the above tabulated series of rabbits there appeared to exist for a brief period of time an acidosis, however, no definite conclusions are considered justifiable.

EXPERIMENTS UPON DOGS

Several groups of dogs were sensitized to either horse serum or egg albumen. When horse serum was used each dog received 5 c.c., 7 c.c. and 10 c.c. of serum intravenously at three day intervals. When egg albumen was used, each animal received 0.25 gms., 0.5 gms. and 1 gm. of dried egg albumen intravenously at three day intervals. Neither of these substances produced a reduction of the carbon dioxide capacity of the dog plasma during the sensitizing injections. Identical results were obtained in the anaphylactic experiments, regardless of whether egg albumen or horse serum was the sensitizing protein. Therefore the results obtained are tabulated together. In the following table is shown the average carbon dioxide capacity of the plasma in twenty-one dogs, previous to and as a result of anaphylactic shock.

TABLE II

Average weight of 21 dogs, 6.5 kg.
Average amount of egg albumen to produce shock in 9 dogs of these series 0.3 gms. per kg.
Average amount of horse serum to produce shock in 12 remaining dogs, 0.5 c.c. per kg.

TIME INTERVALS	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. OF PLASMA
Before injection	52.8
15 minutes later shock dose of protein	
2 hours later	25.1
6 hours later	36.1
24 hours later	48.7

In this table is shown a great decrease of the alkali reserve of the plasma during acute anaphylactic shock in a series of 21 dogs. In several of the animals the carbon dioxide capacity dropped, in two hours' time, to 10 volume per cent. Five of the dogs died within the first six hours. Each of the five animals

showed a carbon dioxide percentage below 20 volume per cent. Two of the animals included in the table showed a drop of only 5 per cent following shock, but clinically both animals were severely shocked. In several of these dogs there was a complete restitution of the carbon dioxide capacity of the blood in six hours after the injection of the shock dose of protein, the minimum CO_2 capacity remaining only a brief time. In three of the animals the blood pressure was also observed, the drop in the alkali reserve being found to begin shortly before the drop in the blood pressure, and the extent of the fall of alkali was directly proportionate to the lowered pressure. When the drop in the alkali reserve was most marked, the animals showed rapid respiration, nausea and vomiting, cyanosis, dilated pupils, and marked prostration.

Having observed a lowered carbon dioxide capacity in the blood plasma of a series of dogs in acute anaphylactic shock, it was advisable to determine how soon after the injection of the shock dose of protein the acidosis begins to develop, and to study the subsequent changes which follow in the alkali of the blood. For this study a number of animals were bled at frequent intervals after receiving the shock dose of protein. The results of these experiments are shown in Table III.

TABLE III

A dog weighing 8 kg., sensitized with 5 c.c., 7 c.c., and 10 c.c. horse serum, intravenously, April 15, 18 and 21, 1919. Upon May 13 anaphylactic shock was produced.

TIME INTERVALS	INJECTIONS	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
May 13th— 9 A.M.		57.6
14th— 9 "		56.7
15th— 9 "		50.0
— 9:10 "	8 c.c. horse serum intravenously	
— 9:17 "		35.7
— 9:42 "		19.6
— 9:47 "		17.6
—10 "	Dog died	

This animal showed a drop in the carbon dioxide capacity of its plasma of 14.3 volume per cent in seven minutes after the injection of the toxic dose of horse serum. In this period the animal showed no noticeable, clinical symptoms of shock. In thirty-two minutes the carbon dioxide capacity had dropped to 30.4 volume per cent with the animal in profound shock. In eighteen additional minutes the animal was dead. This experiment showed a very early and rapidly progressive acidosis in acute anaphylactic shock.

The following experiment shows more clearly the progress of the acidosis after acute anaphylactic shock as the animal was bled at shorter intervals.

A dog, weighing 7.8 kg., was sensitized April 15, 18 and 21, with 5 c.c., 7 c.c. and 10 c.c. horse serum intravenously. On May 15 the dog was rendered anaphylactic. The alkali reserve of the blood was estimated two days previous to the shock to determine the stability of the carbon dioxide in this animal. In order to bleed the animal at frequent intervals a trochar and cannula was inserted into the jugular vein previous to the injection of the horse serum. (See Table IV.)

In three minutes after the shock dose of protein this dog began to show a drop in the alkali reserve of the plasma and continued to decrease rapidly for

TABLE IV

TIME INTERVALS	INJECTIONS	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. PLASMA
May 13th— 9 A.M.		58.3
14th— 9 "		57.6
15th— 9 "		55.7
— 9:10 "	4 c.c. horse serum intravenously	
— 9:13 "		53.4
— 9:18 "		42.1
— 9:30 "		30.4
— 9:45 "		28.7
—10 "		20.2
—12 "		25.2
— 3 P.M.		35.2
— 6 "		45.3
May 16th— 9 A.M.	dog apparently well	54.8

about two hours until the dog reacted favorably to the shock, when the alkali of the blood began to increase and became normal in less than twenty-four hours. The acidosis in this animal was apparent before the development of clinical evidences of shock. In order that the frequent readings necessary to follow these changes would not influence the result of the experiment, only small quantities of blood were withdrawn and 0.5 c.c. plasma used for the tests. The proportion of plasma to the cell volume of the blood was noticeably decreased as the animal became more deeply shocked and greater quantities of blood had to be withdrawn to obtain sufficient plasma for the determinations.

EFFECT OF ALKALINE TREATMENT

Alkaline treatment has been shown to influence favorably toxemic and surgical shock, and since a marked and rapid acidosis is associated with acute anaphylactic shock, it was considered advisable to determine the effect of the preliminary administration of alkali upon this form of shock.

TABLE V

This table represents the average figures of 10 dogs.
Average dog weighed 6 kg.
Average amount horse serum given to produce shock, 1 c.c. per kg.

TIME INTERVALS	INJECTIONS	C.C. OF CO_2 CHEMICALLY BOUND BY 100 C.C. OF PLASMA
May 18th		55.7
19th	2 gm. sodium bicarbonate	58.6
20th	do	62.4
21st	do	67.2
22d	do	73.9
23d	do	79.7
24th—9 A.M.		80.6
10 minutes later	1 c.c. horse serum per kg.	
2 hours later		35.3
6 hours later		40.9
24 hours later		58.4

From Table V it can be seen that in dogs receiving alkaline treatment there is usually a decided drop in the carbon dioxide combining power of the serum following acute anaphylactic shock, but the minimum rarely reaches a point below 25 volume per cent, which was frequently observed in the untreated

dogs and was considered dangerous to the life of the animal. Of the ten dogs treated with alkali only one died, while in the twenty-one dogs not so treated five died. Several of the alkali-treated animals showed severe anaphylactic shock, but their recovery was rapid and their respiratory and circulatory symptoms less severe.

GUINEA PIG EXPERIMENTS

The beneficial effects of the administration of alkali to dogs in anaphylactic shock having been observed and as the practical application of the alkaline treatment of toxemic shock would be of considerable importance, if effective, a similar study was instituted in guinea pigs. Ninety-four guinea pigs were sensitized by the intraperitoneal injection of 0.1 c.c. horse serum. After a sensitizing period of eighteen days, these animals were used for the tests which are tabulated below.

TABLE VI

NUMBER OF GUINEA PIGS	AVERAGE (GMS) WEIGHTS	AMOUNT OF $NaHCO_3$ INJECTED	TIME BETWEEN ALKALI AND SERUM INJECTIONS	AMOUNT OF SERUM	PERCENTAGE MORTALITY	AVERAGE TIME BEFORE DEATH	GENERAL CHARACTER OF SYMPTOMS
10	278	none	———	0.05 c.c.	80	30 mins.	Two animals had severe symptoms of shock while others showed little or none.
10	294	none	———	0.1 c.c.	100	12 mins.	All animals had severe and typical symptoms of anaphylaxis.
10	268	none	———	0.2 c.c.	100	10 mins.	Typical symptoms of anaphylaxis.
8	287	none	———	0.3 c.c.	100	11 mins.	Typical anaphylactic deaths.
14	310	0.1 gms.	10 mins.	0.1 c.c.	85.7	17 mins.	Two animals failed to show symptoms. Twelve died.
14	285	0.3 gms.	12 mins.	0.1 c.c.	78.5	19 mins.	One animal normal. Others showed varying degrees of shock. Ten died.
14	271	0.4 gms	20 mins.	0.1 c.c.	85.7	18 mins.	Symptoms severe. Three recovered and twelve died.
14	290	0.3 gms.	18 mins.	0.3 c.c.	92.8	14 mins.	Symptoms severe and typical but death somewhat delayed. Thirteen died.

In Table VI are shown the composite results upon the experiments of ninety-four guinea pigs, showing the effects of alkaline treatment upon acute anaphylactic shock. The table gives the average number and average weight of the animals; the amount of alkali administered; period of time intervening between the administration of the alkali and the injection of the shock dose of serum; the variations in the amount of the dose of the serum in each series of animals, to determine the approximate minimum lethal dose and the percentage mortality. The length of time necessary to produce death and the symptoms produced after the administration of the toxic dose of the protein is shown. Of the series of animals receiving 0.05 c.c. serum 80 per cent died, while the animals receiving 0.1 c.c. of serum showed a mortality of 100 per cent. The group of animals receiving 0.2 c.c. and 0.3 c.c. of serum showed 100 per cent mortality. These four groups of thirty-eight animals received no alkaline treat-

ment, being used to determine the minimum lethal dose of the foreign serum. This was found to be between 0.1 c.c. and 0.5 c.c. As 0.1 c.c. of serum killed 100 per cent of the animals, this dose was used as the amount of serum for several series of guinea pigs treated with sodium bicarbonate. Of these fourteen pigs were given 0.1 gms. sodium bicarbonate intravenously and in an average period of ten minutes, 0.1 c.c. of horse serum was injected intravenously. An average of 85.7 per cent of these animals died, showing a decreased mortality of 14.3 per cent over the untreated animals. Another group of animals received 0.3 gms. of sodium bicarbonate intravenously and in twelve minutes were given 0.1 c.c. horse serum, with a mortality of 78.5 per cent. Another group of sensitized pigs received 0.4 gms. of sodium bicarbonate and the same amount of horse serum as the previous animals, with a mortality of 85.7 per cent. A fourth series of pigs were given 0.3 gms. of alkali and 0.3 c.c. of horse serum with little protection, as 92.8 per cent died.

The results seemed to show that only slight protection is derived from the preliminary alkaline treatment of the sensitized guinea pigs in acute anaphylactic shock. The mortality of the animals remained high even if large doses of sodium bicarbonate were given.

CONCLUSION

1. Rabbits are unsatisfactory for the study of the changes in the carbon dioxide capacity of the plasma in acute anaphylactic shock, due to the marked normal variations from day to day in the carbon dioxide combining power of their plasma and because anaphylaxis in rabbits is very frequently not an acute shock.

2. Acute anaphylactic shock in dogs is associated with an immediate and progressive acidosis. The acidosis appears before the onset of recognizable clinical symptoms of shock. When the carbon-dioxide capacity of the blood plasma falls below 25 volume per cent the animal usually dies. The acidosis is quickly relieved after shock if the animal survives. The alkali reserve of the plasma is restored to normal in less than six hours.

3. The administration of sodium bicarbonate to dogs before anaphylactic shock has an apparent beneficial influence upon the recovery of the animals. However, it will not always prevent death even though the alkaline reserve of the plasma is restored to normal or above.

4. Alkaline treatment of guinea pigs preliminary to acute anaphylactic shock reduced the mortality 16.7 per cent in a series of forty-two animals treated with relatively large doses of sodium bicarbonate intravenously and a minimum lethal shock dose of the sensitizing protein.

THE ETIOLOGY OF SCARLET FEVER*

III. The Alkali-Producing Organisms in Scarlet Fever

By R. W. Pryer, Dr.P.H., Detroit, Mich.

THIS paper is a continuation of the work started in 1917 in the Bureau of Laboratories of the Detroit Health Department, and which has been briefly reported on in two previous papers.[1, 2]

The results of some rather intensive work in this laboratory for the past year have been so suggestive that it is felt that further report should be made, in order, if possible, to stimulate others along this line of endeavor, and to see whether or not this organism is directly connected with the disease either as the infecting agent or as an associated organism. Practically the only other reported work on the bacteriology of scarlet fever that shows any similarity to ours is that reported by Cantacuzene[3] in 1914. It is possible that he is working with the same organism that we are although he makes no mention of spore production which is one of the distinctive characteristics of our cultures. There are many things about these cultures that lead us to feel certain that they are not ordinary bacteria, among them being their growth on an exceedingly alkaline meat-free medium, their unusual spore production and their appearance at certain stages as revealed by the differential stains.

I think we are safe in saying that if this organism does not come into the higher bacteria group that an alkali-producing, spore-bearing coccus is something new.

However, the possibilty of this organism falling into some division of the so-called higher bacteria group does not by any means exclude it as a possible cause of scarlet fever. Indeed it rather strengthens the chain because of two things we know about the disease.

A fairly regular blood picture in scarlet fever is a gradual increase in the eosinophile count usually starting after the total white cell count begins to fall toward normal. Much the same thing holds true in some other disease of nonbacterial etiology and particularly those caused by the protozoa.

Dr. V. C. Vaughan who has been making an extensive study of the epidemiology of scarlet fever called my attention to the fact that indigenous scarlet fever was practically unknown in the tropics and suggested trying lower temperatures than were ordinarily used, having in mind the work on the cultivation of certain of the protozoa particularly leishmania in kala-azar, and the trypanosomes.

The organisms which we have isolated and reported on previously, produced alkali in all sugar media. There are apparently several groups of these

*From the Bureau of Laboratories, Detroit Health Department.

alkali-producing organisms which can be found in this disease, and we have made a rough attempt to classify them into groups according to certain definite biological characteristics.

Group one and group two have certain peculiarities which make their classification within certain limits rather easy. Since the organisms which we classify as group one have been found only in scarlet fever, we will confine ourselves very largely to a discussion of this group.

Group one. The organisms which we classify as belonging to group one are the same as the large coccus forms reported from this laboratory in previous papers. No mention has been made before of the spore production but after a varying time in subculture on ordinary media the spore production is prompt and regular. These cultures produce alkali in sugar media and are characterized by a peculiar amin odor. They have been isolated from the blood and from the throat but most frequently from the tongue. Although our number of isolations is low compared with the number of cases examined, we feel that this organism is worthy of much study because of its peculiarities. In spite of the great number of other cases examined, we have never found it in anything but scarlet fever. There are several methods which may be used in isolating this organism but two only need be mentioned.

The first is by streaking on blood agar plates and picking colonies from these. It is necessary to pick a large number of colonies since the growth on this medium is not very characteristic. These organisms grow rather slowly at first. The colonies have a regular, definite edge; are flattened and semiopaque. After a number of days on this media they tend to flatten out and become almost invisible so that in the mixed culture that we get in this way, isolation is difficult.

The second method is by the use of a modified Sabouraud's agar. The formula that has given us the best results is as follows:

Peptone	10 grams
Agar	15 grams
Sucrose	100 Grams
Water	1000 c.c.

After solution 50 c.c. of approximately normal sodium hydrate solution is added per liter and 1.66 c.c. of the stock solution of Brom-thymol blue. This stock solution is made by dissolving $\frac{1}{10}$ gram of the dry dye in 15 c.c. of water containing 3.2 c.c. of 20th normal sodium hydrate. The hydrogen-ion value of this medium as determined by the potentiometer is about P_H 9.8. Practically none of the ordinary bacteria are found to grow at all on this medium. However, we have carried cultures of this group on this medium for over a month, although growth is not as abundant or as rapid as on a less alkaline medium. Swabs from the tongue in acute cases of scarlet fever are planted on slants of this medium and incubated at 32½° C. for from two to seven days, and then kept at room temperature (approximately 25°) for about three weeks, occasionally plating on the same medium and watching for typical semiopaque blue colonies. These colonies which require two or three days to develop in good shape are transferred to slants of media of

approximately the same composition, but containing a smaller amount of alkali; the P_H value of these media being 7.4. After 24 hours' incubation at 37½° all the tubes are examined and any showing acid production with apparently pure culture are discarded. The alkali-producing cultures were reserved for further study and the acid tubes that showed a mixed culture were replated. By use of this method, we have been able to isolate the spore-bearing, alkali-producing organism in eight out of forty cases of scarlet fever, while material from fifty other cases including diphtheria, tuberculosis and normal people studied at the same time and under identical culture conditions failed to yield any organism of this type.

THE ORGANISM

The vegetating cell runs fairly uniform in size whatever media is used and whatever temperature at which the culture is grown. Growth will occur at all temperatures between 25° and 42° C. with the optimum temperature lying between 30 and 40°. The limits of size are up to five microns in diameter, and with an average size of about 3 to 4 microns. The actively growing organism is nearly round although in older cultures many apparently coccus-bacillus forms may be seen. The orientation is somewhat suggestive of the staphylococcus and in making a smear in a drop of water on a slide the organisms tend to become evenly distributed in the suspension. Spore production occurs best on blood or nutrient agar at 37½° C. Ordinary incubation temperature is the best whatever medium is used although spore production will occur at 32½° C., but is limited or lacking entirely when grown at lower temperatures. On the meat-free carbohydrate media few spores are found in comparison with the growth on richer media. The spores vary in size within wide limits and the larger ones are frequently lying free and about the same size as the organism itself. Three facts of interest stand out in the study of these spores.

First their comparatively great heat resistance. Two hours' boiling of the suspension in distilled water is not sufficient to kill; the suspension being heated in a test tube within a covered pail. We have had a few suspensions that have withstood a temperature of 110° in the autoclave, but in no instance have we had growth after raising the temperature to 112°, the autoclave being turned off when a temperature of 112° is indicated and allowed to cool down as quickly as possible.

Second, when cultures are made of this heated suspension there is frequently no visible growth after 24 hours in the incubator. However, if a sterile platinum wire is rubbed over the surface of the slants and the slants are returned to the incubator a heavy growth will occur in about eight hours.

Third, no spore can be demonstrated when these cultures are grown in symbiosis with other organisms. For example, the mixture of these organisms with staphylococcus after twenty-four hours on any medium tried show no spore by staining or heat testing methods.

The organism stains readily with the basic aniline dyes, but not with the acid ones. Its behavior to the Gram stain is uncertain but it probably must be classified as Gram-negative. In this connection, it is interesting that

gentian violet in concentration of 1 to 100,000 in solid media completely inhibits growth. With differential stains such as Giemsa's or some of the modifications of the Romanowsky stain in young culture, i. e., freshly isolated cultures, a beautiful contrast stain can occasionally be obtained. The ends of the organism take a deep azure color while the center area is unstained. Within this unstained area is a definite red staining body which closely resembles a nucleus, although it is possible that it is the starting of the spore.

In symbiosis with the staphylococcus, this organism is easily Gram-negative in contrast to its ordinary indifference.

The general appearance of the colony is much the same whatever medium is used. Regular edges, flat dull surface and in older cultures a flattening out and at first glance it looks as though there was no growth on the tube. In all cases there is a slight sticking to the media and the growth is somewhat viscid although nothing like that of the Friedländer pneumobacillus for instance.

The growth in broth and liquid media in general is slow. There is no pelicle formation but after two to three weeks a slimy deposit begins to form in the bottom of the tube. On Loeffler's medium growth is poor.

In litmus milk these cultures grow slowly, but after about three weeks there is a definite production of alkali. There is apparently no acidity shown at any time and no change of any importance other than the gradual production of alkali. Since these organisms grow best on the surface of the solid media, the carbohydrate work has been done in two ways, one set of cultures being planted on a modified Sabourauds agar containing peptone but no meat, and 2 per cent of the carbohydrate which we wish to test.

Another set has been grown in ordinary meat extract broth to which two per cent of the sugar has been added. In both these cases sterilization has been carried out by the intermittent method. The growth and consequently the alkali production is much more rapid on the solid media than in the fluid media.

The initial reaction of the medium is a matter of considerable importance since practically no growth occurs on media in which the P_H value is below 6.8.

We have adjusted the reaction of our media in this carbohydrate work so that after sterilization the P_H value is approximately 7. In all cases in all the sugars tested there is never any acid formation.

We have tested these cultures on the following carbohydrates: glucose, sucrose, lactose, maltose, mannite, dextrin, levulose and zylose.

The amount of alkali produced in any case is not high, the maximum amount observed being a final reaction of P_H 7.8.

In this connection it is interesting to note that alkali production is more abundant and very much more rapid with sucrose than with any other sugar.

The optimum P_H value for growth of this organism is about 7.3 to 7.5 although growth will occur at values as high as 10. On media with a P_H value below 7, growth is uncertain. There is slight growth on media of the following composition; 10 per cent sucrose, 1½ per cent agar, and with alkali added to bring about a reaction of P_H 7.3.

To summarize somewhat the cultural characteristics of this organism the growth is strictly aerobic, optimum temperature 30 to 40, a spore-producing, alkali-producing, coccus-like organism which will grow on very alkaline media and a medium very deficient in meat or meat products.

In a great many respects this organism resembles some of the yeast family, although no budding forms have ever been observed, and multiplication as far as can be determined is by cell division.

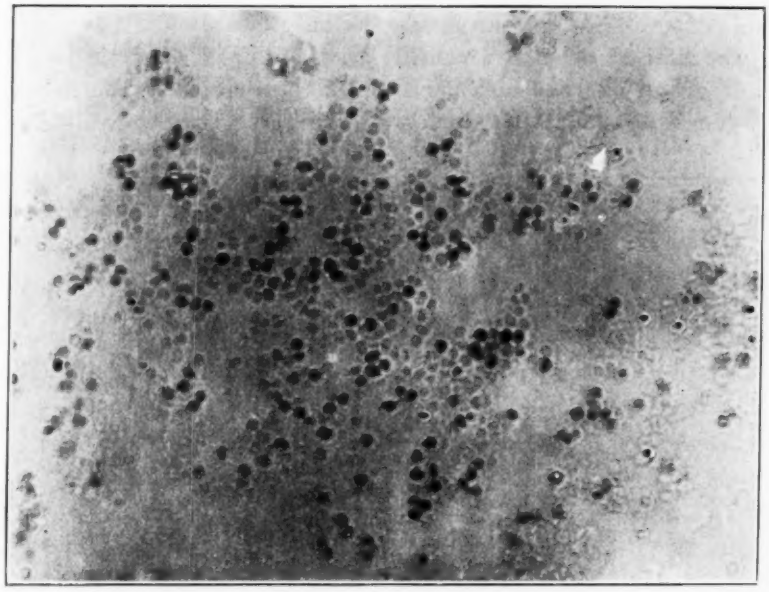

Fig. 1.—Huntoon's spore stain Group I. x1500.*

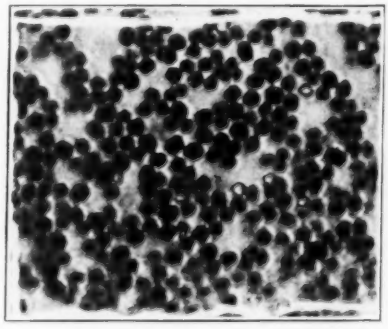

Fig. 2.—Gram's stain Group I. x1500.

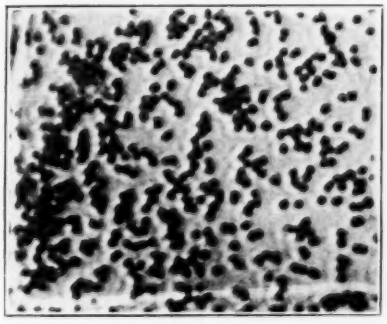

Fig. 3.—Gram's stain Group II on alkaline sucrose media. x1500.

The pathogenicity has been tested on guinea pigs, mice and rabbits. Freshly isolated cultures injected intraperitoneally into mice or guinea pigs will kill in from one to three days. Considerable evidence has been accumulated that this organism when isolated from the blood of an animal dying

*The writer desires to express thanks to Dr. F. G. Novy, Professor of Bacteriology, University of Michigan, for these photographs.

following injection is of somewhat different morphology than is the culture injected. However, this phase of the work must wait for further investigation before a full report can be made.

The pathogenicity is soon lost and so far at least we have been unable to exalt their virulence. So far, we have been unable to demonstrate pathogenicity for rabbits either on intraperitoneal injection or intravenous injection.

SEROLOGY

The production of agglutinating serum in rabbits takes place rather slowly. The titer of the best serum we have obtained so far is 1 to 800. All cultures of group one are agglutinated in approximately the same dilution by this serum. Agglutinating work with the serum from convalescent scarlet

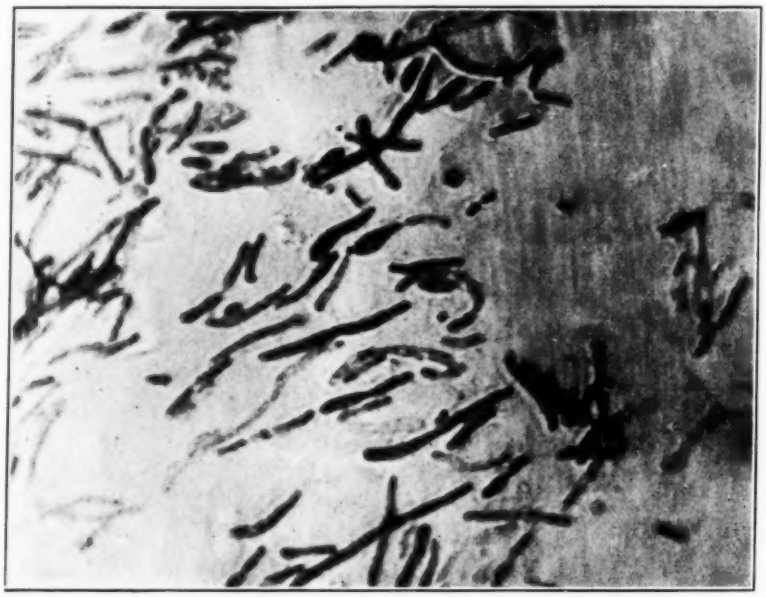

Fig. 4.—Gram's stain Group II, 8 hours on blood agar, x1500.

fever patients has not progressed far enough as yet to justify drawing any conclusions. There is what is seemingly a false agglutination which occurs when the diluted serum and organism suspension are mixed; and a complete report on agglutination work must wait until some later time. Group 2. The organisms which we classify as belonging to Group 2 are included in this paper because of the fact that under certain cultural conditions their morphology resembles somewhat that of group one. This culture also produces alkali in all sugars tested, but no spores have been observed. On the modified meat-free agar these organisms tend to maintain a coccus form of usually less than a micron in diameter. They are frankly Gram-negative in whatever form they may be.

The microphotograph (Fig. 3) gives a fair idea of their size and appearance when carried in culture on this medium at a temperature of 32½°.

When these cultures are transplanted from this media on to blood agar slants or plates, and these are incubated at 37½° a striking change occurs in these organisms within about eight hours. Examination at this time usually shows a tremendous increase in size and varying from a large coccus to long filaments, the largest filament that we have observed measuring 57 microns in length.

If these blood agar cultures are retained in the incubator for about 24 hours longer, the majority of the organisms have changed back again to the small coccus form and if transplanted back on to the alkali meat-free agar again show no filament.

The differential stain on these organisms in what we call the monster form show nothing very definite in the morphology or in the cellular arrangement. Multiplication in these cultures is also by cell division.

When these are carried on ordinary nutrient agar at a temperature of 37½° their general morphology resembles Group 1 so closely that mistakes might occur. The separation of Group 2 from Group 1 is fairly easy from the fact that Group 2 produces no spores and does, under the conditions outlined above, produce monster forms. The agglutinating serum prepared for members of Group 1 shows absolutely no agglutinating action when tested against group two. Group two has been isolated more frequently from scarlet fever than from other conditions, but has been found in at least one case in a normal individual.

We have several other cultures that are frank alkali producers and in general have cultural characteristics very similar to those of Group 1 and Group 2. Morphologically they are Gram-negative, rather small diplococci with which we have been unable to produce the monster forms, and in which we have been unable to demonstrate spores at any time.

There are several different organisms that in general morphology resemble Group 1 very closely. However, they can soon be ruled out by the fact that they either fail to produce alkali or else produce a colored growth and in practically every instance produce no spores. Members of these undetermined groups make up the groups of large cocci that are frequently seen in the examination of cultures for the diphtheria bacillus.

In conclusion a large coccus-shaped organism which in size resembles somewhat a yeast has been isolated from scarlet fever patients and has not as yet been found in other conditions.

This organism is characterized by alkali production in all sugars, abundant spore production and typical amine odor. The spores are very resistant to heat, withstanding at least two hours' boiling, but are usually killed by a temperature of 110° in the autoclave.

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STUDIES ON THE RESISTANCE OF THE RED BLOOD CELLS*

III. The Relation of Cholesterol to the Resistance of the Red Blood Cells to the Hemolytic Action of Sapotoxin

By Chas. H. Neilson, M.D., and Homer Wheelon, M.D., St. Louis, Mo.

TWO theories are prevalent concerning the union of hemoglobin with the other constituents of the red blood corpuscle. The first and older theory holds that the hemoglobin is held or surrounded by a wall which is not permeable to hemoglobin. The modern theory, "Theory of Jellies," defends the belief that the hemoglobin is held in the corpuscles because of its chemical union with the stroma. Such a condition holds for all the other cells. Moreover, puncture or cross-section of the red cells does not permit of extrusion of the hemoglobin from the cell or its two halves. If the hemoglobin is set free in the corpuscles it will crystallize, showing that it must be prevented by some means from crystallizing in the normal cell. Further, the concentration of hemoglobin in the mammalian corpuscle is greater than the solubility of oxyhemoglobin in an equal bulk of water. Therefore, it seems more nearly correct to assume that the hemoglobin is maintained in a loose chemical union with the stroma of the corpuscle than to assume that it is confined within a bag or cell membrane.[39] Moreover, the corpuscles will not allow many of the salts to pass although they are easily penetrated by water. For instance, urea is taken up and distributed equally between the red cells and the plasma: its solution exerts no osmotic pressure upon the cells. The corpuscles behave in urea solutions of all concentrations exactly as in distilled water, that is, they give up their hemoglobin to the urea solution. Such does not occur if the urea is added to an isotonic solution of sodium chloride. Again, ammonium chloride readily penetrates the red blood cell and hemoglobin is forced into solution even if the ammonium chloride is added to isotonic saline: ammonium chloride, therefore, has a direct poisonous action upon the corpuscles.[1]

Certain agents are capable of liberating the hemoglobin from the red cells whether we believe that these cells are little bags containing hemoglobin or organized jellies. Such liberation of hemoglobin may result either because of physical[24] or chemical processes.[18, 39] That is, the hemoglobin may be freed from the cells by toxins such as snake poisons,[9, 18, 74] bacterial processes,[18, 68] plant poisons,[48, 55, 75] by small amounts of alkalies, water, ether, chloroform, salts,[1, 10, 23, 24, 26, 37, 40, 56, 71, 72, 73] fatty acids,[41] mechanical and thermal injuries,[36, 49, 50] and disease.[11, 12, 43, 51, 52, 57, 59, 60, 63, 64]

In certain respects the erythrocytes do act as semipermeable membranes. They allow water to pass in and out without changes in volume. However,

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the red blood cells are not permeable to salts. Some certain anions, Cl′ CO_3'', SO_4'', pass in, but this can only occur providing that other ions pass out in compensation, on the other hand, the cations do not pass through at all. The quantity of CO_3'' ions in the erythrocytes to a marked degree influence the permeability of the cells.[1, 4, 39] Solutions with equal molecular concentration with the red blood cells have no power to draw water from the cells; they are therefore isotonic or possess the same osmotic pressure. Reversible osmotic phenomena can proceed for a considerable distance in either direction without injury to the cells; on this principle, a method for determining the resistance of the cells has been worked out by exposing them to a series of salt solutions of graded strength. Such a method, one may believe, determines only the osmotic resistance of the red cells. The surrendering of the coloring-matter by the red cells is not always complete in any given salt solution. This condition is explained by Hamburger[24] by supposing that individual corpuscles have different degrees of resistance. This observation is also true whether the cells are maintained in their normal serum or when washed. When it is recalled that the life of the red cell is comparatively short and that a certain number are being continually destroyed by the spleen and liver, it seems that such an explanation is justifiable, for the resistance of the red cells must to some degree depend upon its vitality.

The theory of membraned cells and osmotic resistance naturally fall together as interpretive measures. On the contrary the action of specific hemolytic agents, animal and vegetable poisons, cannot be easily explained by assuming osmotic changes responsible for the destruction of the cells. The stroma of the red cells freed from its hemoglobin content is toxic and causes intravascular clotting. This stroma toxicity, in all probability, is responsible, or at least leads in the hemolytic processes of certain diseases which cause a breaking down of the red cells, i. e., malaria, syphilis and hemolytic jaundice. The stroma of the normal cell, or when in combination with hemoglobin as it exists in the normal cell, is perfectly inert.

Certain vegetable glucosides such as the saponins and animal secretions (snake, insect and reptilian poisons) act as direct or perhaps specific hemolytic agents. The hemolytic action of certain bacteria is well known. In this relation it is interesting to note that the pressure of carbon dioxide acts as a protective agent to the red blood cells against the action of various hemolytic sera.[68] Experimental work with the saponins and anisotonic salt solutions have shown that various degrees of altered red cell resistance occurs in many of the common diseases. In cases of experimental anemia the resistance of the erythrocytes is raised to an enormous degree. For instance poisoning by phenylhydrazin so increases the resistance of the red cells that they may be placed in distilled water, even after having been washed free from the serum, without being laked. This increased resistance is associated with an increase in the stroma of the red blood cells—pachydermia.[4] In hemolytic icterus the resistance is low; in obstructive jaundice raised. In pernicious anemia the resistance is said to be raised while in hemoglobinuria—

paroxysmal hemoglobinuria—there is apparently no change in the osmotic resistance but a diminution of both the thermal and chemical resistance.

Various workers following Hamburger's method (anisotonic salt solutions) have reported studies upon the resistance of the red cells in various pathological conditions.[10, 24, 26, 27, 37, 59, 63, 64, 72, 73] The results obtained by this method may be summarized as follows. It is increased by the addition of hydrogen, nitrogen, carbon monoxide, carbon dioxide and acids, and is diminished by traces of alkalies and oxygen. Clinically it has been found that during the course of typhoid fever, pneumonia, other acute infections, pregnancy and lactation, some malignant growths, obstructive jaundice, eclampsia, diabetes, cardio-renal disturbances and secondary anemias that the isotonic tension may be increased. A lowered resistance is found in hemolytic jaundice, pernicious anemia, certain malignancies and chlorosis. (Table I).

TABLE I

CHOLESTEROL AND THE RESISTANCE OF THE RED BLOOD CELLS TO SAPOTOXIN

		RESISTANCE TO SAPOTOXIN			CHOLESTEROL			RESISTANCE TO SALT SOL.	
CONDITION	NO.	RESIST.	HB	VARIATION FROM AVERAGE NORMAL	INC.	NORM.	DEC.	INC.	DEC.
Normal	185	1:13,937	91					NORM.	0.46
Pregnancy	35	1:11,271	89	+ 2,666	+	+	−	+	−
Malaria	23	1:14,380	86	− 443	−	+	−	−	+
on quinine	32	1:15,242	89	− 1,305	−	+	+	+	+
Normals	5	1:13,944	88	− 7		+	−	−	−
Tuberculosis	24	1:12,375	82	+ 1,562	+	−	−	+	−
and Lues	2	1:13,875	68	+ 62	−	?	−	−	−
and Pregnancy	2	1:10,250	85	+ 3,687	+	−	−	+	−
Typhoid	19	1:13,513	85	+ 427	+	+	+	+	+
Jaundice									
All types	31	1:12,685	79	+ 1,252	+	+	+	+	+
Obstructive	15	1:10,033	84	+ 3,904	+	−	−	+	−
Hemolytic	5	1:18,600	81	− 4,663	−	−	+	−	+
Anemia									
Pernicious	8	1:15,063	35	− 1,126	−	−	+	−	+
Cardiorenal	8	1:12,312	60	+ 1,625	+	−	−	+	−
Syphilis	7	1:15,750	58	− 1,813	−	+	+	?	?
H. Jaundice	4	1:17,125	58	− 3,188	−	−	+	−	+
Carcinoma	5	1:12,190	51	+ 1,747	+	?	−	+	−
Kidney									
Nephritis	12	1:13,958	79	− 21	+	+	−	+	+
Cardiorenal	5	1:14,500	84	− 563	−	+	+	+	+
Eclampsia	2	1:10,500	83	+ 3,437	+	−	−	+	−
Uremia	5	1:14,050	85	− 113	?	?	?	?	?
Diabetes	2	1:13,079	88	+ 858	+	+	−	+	−
Malignant growths	19	1:13,500	78	+ 437	+	+	−	+	+

The above information concerning cholesterol and resistance of the red cells to anisotonic salt solutions is drawn from the literature. Our cholesterol determinations accorded with those in the literature, hence, it was not deemed necessary to tabulate these results numerically.

In previous communications we reported the results of some two thousand experiments on the resistance of the red blood cells in health and various of the common diseases to the hemolytic action of sapotoxin. The result of this work is summarized in Tables I and II. In brief our technic for making resistance determinations was as follows:[81] A given amount of whole

blood, 20 c.mm., was exposed for five minutes at a constant temperature, 25° C., to various strengths of sapotoxin solution. Usually a set of 4 small tubes each containing 1 c.c. of the sapotoxin solution in graded dilutions was used to make the primary reading. If the point of minimal hemolysis did not occur in the first series of dilutions a set of higher or lower concentrations were tried out. The solution in which a minimal degree of hemolysis occurred was taken as the resistance point of the red cells. With this technic, the details of which appeared in our first paper, it was found that 185 determinations on normal whole blood gave an average resistance reading in a 1:13,937 sapotoxin solution. It was also shown that the removal of the serum and blood fluid from the red cells resulted in a marked loss of resistance (Table II). That is, the presence of blood fluids about the red cells in some way acted to increase red cell resistance. In disease it was found that the red cell resistance was increased in cases of secondary anemia, cardio-renal disturbances, diabetes at times, eclampsia, obstructive jaundice, some cases of malignant growths, pregnancy, some cases of pneumonia, and pulmonary tuberculosis. Red cell resistance was found diminished in cases of pernicious anemia, hemolytic jaundice, and in syphilitics on potassium iodide. The resistance of the red cells in certain cases of diabetes, some secondary anemias, malaria without quinine treatment, some cases of nephritis, lues on mercury and typhoid fever, was either normal or but slightly altered (Tables I and II).

TABLE II

RESISTANCE OF WASHED AND UNWASHED RED BLOOD CELLS TO SAPOTOXIN

	WASHED			UNWASHED			1:1 WASHED CELLS AND SERUM	
	NO	HEMOLYSIS	HB	NO	HEMOLYSIS	HB	NO	HEMOLYSIS
Normal	12	1:37,375	86	185	1:13,937	90	10	1:14,050
*Syphilis	21	1:41,357	81	34	1:14,699	85	4	1:16,437
Pregnancy	5	1:37,100	87	35	1:11,271	89	3	1:12,050
Jaundice	4	1:36,750	85	31	1:12,685	80	4	1:12,564
Tuberculosis	3	1:35,000	87	24	1:12,375	81	3	1:13,025
Average	45	1:38,900	85	309	1:13:472	85	24	1:13,625

Difference between washed and unwashed cells = 25,428 points.

Table showing degree of difference in the resistance of red blood cells when washed and when in contact with their normal fluids. No, number of determinations; Hemolysis, strength of sapotoxin solution required to cause a minimal hemolysis in 5 minutes; Hb., Hemoglobin percentage.

*Untreated.

In general our findings are in accord with the results obtained by the use of the anisotonic salt solution methods for the determination of red blood cell resistance (Table I).

In 1902 Flexner and Noguchi[18] published a series of experiments which throw considerable light upon the chemical nature of hemolytic actions. They found that red blood cells freed from their serum by continued washings in normal saline failed to lake when suspended with cobra poison in an isotonic sodium chloride solution. On the other hand washed corpuscles to which a small amount of serum had been added were immediately destroyed. They also noted the same occurrence with other poisons, tetanus toxin, salanin and saponin and justly concluded that some substance was undoubtedly present

in the serum which made it possible for cobra poison to act on the hemoglobin of the corpuscles. Keyes demonstrated that lecithin is the activator of snake venoms uniting with it to form a toxic cobra-lecithin; lecithin could be substituted for serum, although lecithin alone would not act as a hemolytic agent.[1, 4]

The chemical function of the phospholipins is that of making, with the sterols (cholesterols), a water containing semifluid, highly reducing, auto-oxidizable, semilipoid crystalline substratum of protoplasm. They also are actively concerned in certain of the phenomena of immunity. However, the "lecithides" are very hemolytic. Lecithin emulsions have the same power to a certain degree. Hence, we may believe, that the phosphatides by combining with toxins, or possibly blood stuffs, influence the power of the latter to unite with and affect the activity of protoplasm. Either phospholipins or glycolipins have the power of binding with tetanus toxin.[39]

Certain metabolic products of insects, fishes, reptiles and crustaceans are also poisonous. These hemolysins of animal origin have their analogues in the saponins of vegetable origin. Lecithin favors saponin hemolysis as in snake poison,[9, 32, 44] while cholesterol inhibits hemolytic activity. The poisoning due to marchella is powerfully hemolytic in its effect, causing fever, jaundice, hemoglobinemia and hemoglobinuria. This poison can be made innocuous by boiling.

Recent experimental work has shown that the blood plasma and red cells are especially rich in cholesterol.[3, 5, 7, 13, 15, 17, 20, 21, 22, 25, 28, 29, 30, 33, 34, 38, 42, 47, 54, 61, 62, 67, 69, 71, 80] Cholesterol is found in an uncombined form in the red cells and as cholesterol esters in the plasma. Bloor,[6] for instance, has shown that lecithin (including cephalin) in the corpuscles is nearly double that in the plasma while cholesterol and total fatty acids are more abundant in the plasma. The amount of lecithin in the corpuscles is about double the amount of cholesterol, but in the plasma the two substances are about equally distributed. He also found that the lecithin-cholesterol ratio is constant in normal blood and in many pathological cases.

Cholesterol is one of the most important of the physiologic substances and plays a great part in certain pathologic conditions. Cholesterol forms a weak molecular union with saponaceous substances, such as digitonin, saponin, and other hemolytic glucosides. It thus protects the red blood cells by neutralization of their toxic action.[1, 4, 39] Red cells are continuously being acted upon by hemolyzing substances and dissolved. Hence, if the rate of destruction becomes greater than that of new formation, varying degrees of anemia must of necessity result. Cholesterol offers protection to the cells against many of these substances, cholesterol also acts to neutralize or check the action of lipolytic enzymes. Cholesterol and certain of its derivatives assist other lipins in giving to cells their power of holding large quantities of water without losing their peculiar semifluid character, and without dissolving. Cholesterol forms one of the most abundant lipins of the brain and occurs in nearly all living tissues. There is evidence to show that cholesterol is the mother substance from which the bile acids are derived; it also probably gives rise to certain lipochrom pigments and possibly to some of the odorif-

erous substances of plants and animals. The total cholesterol in the blood serum of normal individuals varies within rather narrow limits: the average amount is from 0.15 to 0.18 per cent.[39] A condition of hypercholesteremia may be brought about experimentally by the feeding of cholesterol or cholesterol-containing foods.[16] A physiologic hypercholesteremia occurs normally during the later months of pregnancy. The cholesterol content of the blood is increased in certain pathologic conditions. In complete occlusion of the common bile duct the cholesterol is high in the blood as normally in the bile. The cholesterol is increased in cases of lipemias with severe diabetes, nephritis, and arteriosclerosis in which case the esters of cholesterol form doubly refractory masses. In certain of these conditions the hypercholesterolemia appears to be associated with a mobilization of other fatty substances in the body. Local deposits of cholesterol occur in atheromatous arteries, white plagues of nephritic retinitis, arcus senilis, exanthemata, old infarcts, caseous material of tubercular lesions, and gall stones. According to Pighini the cerebro-spinal liquid of syphilitics generally contains more cholesterol than normal. The blood cholesterol is usually normal or diminished in pulmonary tuberculosis, certain of the anemias, hemolytic jaundice, etc.[2, 5, 6, 7, 9, 13, 14, 15, 21, 30, 35, 41, 43, 45, 47, 67, 76, 78, 79, 81]

Certain acids formed by the oxidation of cholesterol are poisonous and possess a toxicity and hemolytic action comparable with some of the snake venoms. Windaus[80] obtained such an acid which was more hemolytic than the bile acids, and as powerful as many saponins in causing red cell destruction. Local necrosis similar to that caused by snake bite followed injections of this acid.

With these points in mind the inevitable conclusion was reached during the progress of our studies in red cell fragility that the lipins of the blood in some way determined the action of the hemolytic agent sapotoxin. As a check upon this conclusion a certain number of cholesterol and resistance tests were made upon the same bloods. In our cases the cholesterol of the blood was found high in the latter part of pregnancy, in obstructive jaundice, one case of mercury poisoning, tuberculosis, certain cardio-renal diseases, certain diabetics, eclampsia, and in some cases of anemia. Cholesterol was found low in cases of hemolytic jaundice, pneumonias associated with jaundice, in certain malignant cases; especially those associated with anemia and cachexia. In typhoid, malaria, anemias in general, syphilis with and without treatment, and tuberculosis the cholesterol findings varied. Resistance of the washed and unwashed cells gave readings comparable to those of the cholesterol content of the blood (Tables I and II). In the one case of chronic mercury poisoning it was impossible to wash the red cells without causing their complete destruction. However the cholesterol content of the blood was found to be 285. Apparently, in this case the high content of cholesterol of the serum alone was sufficient to prevent breaking down of the red cells. A similar condition was frequently observed while working with syphilitic bloods, especially with those which had been treated with mercurials. Our

findings relative to the cholesterol of the blood are comparable to those reported by other workers for similar diseases.

As previously shown[81] the removal of the blood fluids from the red cells results in a marked lowering of the resistance of the red cells to sapotoxin solutions. However the same washed cells diluted 1:1 with their own serum demonstrated a practically normal resistance. Similar observations were made on diseased bloods the results of which are shown in Table II. Numerous observers have shown that the blood fluids contain cholesterol. Hence, if as has been assumed the cholesterol content of the blood fluids acts as a protective agent against the hemolytic action of sapotoxin, then variations in the cholesterol content of the blood fluids about the cells should result in variations in the resistance of the red cells. That such a condition does subtend is shown by the following considerations.

The red cells from 4 cases of obstructive jaundice when washed gave an average resistance of 1:36,750. The average resistance of 12 determinations made on normal washed cells was 1:37,375. However the red cells in whole blood show a great degree of resistance in cases of obstructive jaundice—1:10,033. Normal washed cells diluted with their own serum and cells in whole blood show a normal degree of resistance—1:13,937. In cases of obstructive jaundice the cholesterol of the blood fluids is increased,[21, 25, 47, 61, 62, 77] hence if the cholesterol content of the serum acts as an antihemolytic agent then a serum containing a high cholesterol content should raise the point of resistance of normal red cells when diluted with such a serum and exposed to the action of sapotoxin. That such really occurs is shown by the following experiment upon two jaundiced cases: Case X, catarrhal jaundice of mild degree and short standing, gave an average resistance of whole blood of 1:12,750. The red cells when freed from their blood fluids gave an average resistance of 1:38,000. The resistance of the same cells when diluted 1:1 with their own serum gave an average resistance of 1:13,750. The cholesterol content of the blood was 168. Case Y, obstructive jaundice of long standing, gave an average whole blood resistance of 1:9,000. The washed cells gave an average resistance of 1:34,000. A dilution of the washed cells with their own serum gave a resistance reading of 1:11,500. The cholesterol content was 292. Dilution of washed cells of Case X with the serum of Case Y which was high in cholesterol content gave an average reading of 1:10,750, an increase of 3000 points in the resistance of the washed cells when surrounded with their own serum. On the other hand, a 1:1 dilution of the red cells of case Y with the serum of Case X, which was lower in cholesterol content, gave an average reading of 1:17,500, or a loss of 6,000 points in the resistance of the cells to sapotoxin. The results of this experiment show that not only the blood fluids as such but also their cholesterol content act in such a manner as to raise the red cell resistance. This protection offered the red cells by cholesterol is, no doubt, of a chemical nature. That is, the hemolytic power of sapotoxin becomes manifest in proportion to the degree of neutralization of its action by cholesterol.[8, 32, 39, 48]

The above considerations lead to the assumption that the increased cholesterol content of the blood in cases of obstructive jaundice is at least in great part responsible for the increased resistance of the red cells to hemolytic substances. This assumption becomes weightier when it is recalled that cases of hemolytic and congenital jaundice show a reduction of the blood cholesterol and also a decreased resistance to the action of anisotonic salt solutions[11] and sapotoxin.

In the anemias Bloor[6] has shown that whenever the percentage of the blood corpuscles drops below one-half of the normal that abnormalities appear in the lipoid content which in the order of their magnitude and occurrence are: (a) high fat in plasma; (b) low cholesterol in plasma and occasionally in the corpuscles; (c) low lecithin in the plasma. Such observations offer no certain evidence that abnormalities in the blood lipoids are responsible for anemia, however, the low value for cholesterol which is an antihemolytic substance, and the high fat fraction which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood may be considered as possible causative factors in the production of anemia.[4, 6, 15, 26, 41, 43, 53, 57] Whether a disturbed lipoid metabolism is the cause of anemia or not, the fact remains that the resistance of the red blood cells varies in proportion to the cholesterol content. Hence, it may be assumed that a normal cholesterol content is essential to the maintenance of the normal resistance of the red cells to hemolytic substances.

SUMMARY AND CONCLUSIONS

The present work, therefore, leads to the conclusion that cholesterol is an important element in the protection of the red cells against the hemolytic action of sapotoxin. Because of this conclusion we feel that a chemical examination of the blood for cholesterol is to be greatly preferred to the more laborious and inaccurate task of estimating the red cell resistance to hemolytic agents. Furthermore, we would emphasize the fact that resistance determinations are of little clinical value save for the differentiation of the two types of jaundice because of the wide range of variations that occur in many of the common diseases.

In brief, our results are as follows: The red cells may be considered as composed of jellies which exist and depend upon their content and peculiar behavior of lipins. The cholesterol, both of the cell itself and about the cell acts as an antihemolytic while lecithin tends to combine with toxins to form lecithides. Such formations react upon the cell to destroy its composition, thereby liberating the hemoglobin from the stroma. Diseases in which the cholesterol content is high show an increased red cell resistance to sapotoxin solutions; those showing a low cholesterol content also show a lessened degree of resistance to specific hemolytic agents. Hence it may be concluded that the cholesterol of the blood in great part determines the degree of resistance of the red cells to sapotoxin solutions.

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LABORATORY METHODS

THE WASSERMANN TEST AND ITS INTERPRETATION*

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I.

INTRODUCTION

JUDGING from the requests for information regarding the Wassermann test which come to this laboratory, it was felt that a paper embracing, first the requirements of the laboratory for correct tests; second, the responsibility of the physician toward this end, and third, the interpretation of the test, would be helpful to many who are concerned in venereal disease control. It was with this view in mind that this paper was prepared.

Many attempts have been made to standardize the Wassermann test. The New York City Department of Health,[1] for example, inaugurated a movement to this effect among New York City Wassermann workers in 1915. It was soon found, however, that there existed so much difference of opinion among the different workers regarding the procedure of the test, that the attempt was finally abandoned. Standard Wassermann procedures have also been attempted by the U. S. Public Health Service[2] and the Massachusetts State Board of Health.[3]† It appears that the standardization of the Wassermann technic, to the extent of its being employed by most Wassermann workers, is a very difficult task. The standardization of the essential requirements of the test without regard to the procedure, however, can be brought about.

This phase of standardization was discussed in a recent paper from this laboratory[4] where a number of requirements were set down as being essential for correct Wassermann tests. These requirements, as well as others affecting the correctness of this test, will be more fully considered below. There are also phases influencing Wassermann results which are largely in the hands of physicians and health officers. Finally, there are numerous questions relative to the interpretation of Wassermann results. Each of these, for the sake of clearness, will be discussed under separate headings.

II

WHAT THE STATE LABORATORY IS DOING TO INSURE CORRECT WASSERMANN TESTS

1. *The Duplication of Wassermann Tests.*—It is evident that the Wassermann test should be performed with the highest accuracy. This particularly applies to the public health laboratory. In a hospital laboratory, if the Was-

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†The Studies on the Standardization of the Wassermann Test by Kolmer and Co-workers (Am. Jour. Syph., 1919-21) have not been completed at the time of preparation of this paper.

sermann results do not check the clinical diagnosis, there is no serious harm; another specimen can be obtained with little difficulty and the test repeated. In a public health laboratory, however, where the specimens come from physicians who are scattered over a wide area, the Wassermann results must necessarily be accepted as practically final. For a public health laboratory to report a positive Wassermann on one free from syphilis is a very grave error indeed. Then again, to report a false negative might result in seriously endangering the health of the community. To overcome both of these possibilities, this laboratory runs two Wassermann tests of varying sensitiveness on every specimen coming for examination. One of these tests is carried out with the alcoholic-extract antigen and ice-box fixation. This procedure is generally accepted to be free from the possibility of picking up false positives. There are, however, isolated cases where the cholesterinized antigen appears to be more sensitive than the alcoholic-extract antigen. A duplicate series of tests are thus carried out with this antigen in order to pick up the occasional weak positive which the alcoholic antigen might miss. And in order to help overcome the tendency of the cholesterinized antigen to pick up an occasional false positive, only three antigenic units are employed, also, instead of ice-box fixation, water-bath fixation is resorted to. The interesting fact regarding these two procedures is that they check practically 100 per cent. Of 8000 specimens examined during the past four months, only five did not check by both methods, or one specimen in 1600.

The employment of two distinct Wassermann procedures of varying sensitiveness for each specimen of blood, in our opinion, goes a long way toward the solution of the problem of correct Wassermann tests. The mere duplication of tests, however, does not necessarily mean that the final results are absolutely correct. What the State Laboratory is doing to render the results of the *individual* Wassermann tests of the highest accuracy, will now be considered.

2. *The Removal of Antisheep Ambocepter from the Patient's Serum.*—Perhaps the main reason why natural amboceptor is not removed from the patient's serum in a large number of Wassermann Laboratories, is because of the tediousness of the task.[5] Prof. Charles E. Simon,[6] a strong advocate of the removal of natural amboceptor from the patient's serum, makes this significant statement regarding this question in a recent paper: "I have reason to assume that there is a tendency to let well enough alone, on the ground that the original technic is time-consuming enough, as it is, and that any tendency to further lengthen the process would be disregarded."

The fact that natural amboceptor introduces a definite source of error in the Wassermann test is well established.[7, 8] If the blood of a syphilitic patient should happen to contain a small number of so-called syphilitic antibodies and a relatively large amount of natural amboceptor, it would be likely to give a negative Wassermann result. Thus positive reactions are continuously apt to be lost, if the natural amboceptor is not removed from the serum.

In this laboratory, the removal of antisheep amboceptor from the patient's serum is part of the routine procedure of the Wassermann test. Our method is fully described in another paper.[9] Briefly, it consists of adding concentrated

sheep-cells to inactivated sera in the proportion of 1 drop to 1 c.c. and permitting it to extract for 10 minutes at room temperature. The sheep-cells are then thrown down by centrifuging at high speed and the clear supernatant serum is ready for use. This simple procedure for removing natural amboceptor has been applied in this laboratory to about 16,000 Wassermann tests, with entirely satisfactory results. It is efficient in its amboceptor removal; it does not cause the serum to become anticomplementary; and its time-consuming element is practically negligible.

3. *Complement and Amboceptor and their Daily Titrations.*—Complement is obtained by bleeding guinea pigs under anesthesia directly from the heart. The blood is placed in the ice box immediately after clotting and the clear serum is drawn off about 15 hours after bleeding. From 3 to 5 pigs are bled daily in order to insure the uniformity of the complement. No complement is employed unless it is practically free from natural amboceptor and possesses a good complement titre.

The amboceptor employed is prepared by immunizing rabbits with sheep-cells, previously washed five times with saline. Healthy rabbits are given three intravenous injections of packed cells at 48-hour intervals. The first injection consists of 1 c.c. and the second and third of 2 c.c. each. Four or five days after the last injection, an amboceptor titre of about 1-2000 is usually obtained.

The sheep cells are obtained once every three days from our own sheep kept for this purpose. In this regard, our advantage over those workers who are obliged to obtain sheep blood from the abattoir is evident. In the latter case, the resistance of the corpuscles to hemolysis is likely to vary from day to day, while in our case this factor is kept practically constant.

The cells are washed four times with salt solution in order to wash them free from serum; and after the fourth, or final washing, they are packed by centrifugation at the same speed for an equal period of time every day. The concentration of cells is in this way kept uniform from day to day. After the cell suspension is made up in its proper dilution for the day, it is filtered through several layers of cheesecloth. This is done as a special precautionary measure in order to remove tiny clots which frequently find their way in the suspension, and which have a tendency to absorb large amounts of amboceptor.

The unit of new amboceptor is determined by preparing a series of dilutions of amboceptor-serum and titrating each with 0.1 c.c. of a 5 per cent suspension of sheep-cells and 0.1 c.c. of 1-10 pooled guinea pig complement (1/10 quantities of classical Wassermann). The dilution of amboceptor-serum aimed at, is one which contains two units of amboceptor in 0.1 c.c. This dilution being determined, it is titrated daily for a week with different pooled complements, in order to establish a permanent amboceptor unit.

Complement is titrated daily to determine the smallest amount which will hemolyze the standard quantity of sheep-cells in the presence of 2 units of amboceptor. This smallest amount is known as the complement unit, and the obtaining of this unit is considered of such importance that it is not left for one worker to determine it. Two workers make complement titrations

and determine the complement unit independently of each other, and these units must check or the titrations are repeated.

The problem of complement fixation, as the name implies, is the problem of the amount of complement "fixed" or "bound" by serum and antigen. This amount, as is well known, is far from being unlimited. An excess of complement can easily obscure the amount fixed by serum and antigen and render a syphilitic serum negative. An insufficient amount of complement, on the other hand, can render a negative serum positive. It is felt, therefore, that the determination of the complement unit with the utmost care is time entirely well spent.

We do not, however, stop with daily complement titrations; we daily titrate our amboceptor as well. This is done not because of the slight changes in the keeping quality of the amboceptor, but in order to insure that no less than 2 units of amboceptor will be employed with the complement and cell suspension of any given day. These amboceptor titrations are carried out toward the end of the fixation periods—just before adding amboceptor and cells to the tests. And as a result of these extra titrations, we have been able to avoid the occasional difficulties which come up in a Wassermann laboratory, due to an unbalanced hemolytic system.

The advantages derived from these daily amboceptor titrations are fully discussed in another paper.[10] Suffice it to say in this connection that these titrations enable one to determine the amount of complement which has deteriorated during the fixation period. We employ 2 units of complement in our tests, but by the end of the fixation period, when amboceptor and cells are ready to be added, we might be dealing with 1¼ to 1½ complement units instead. In other words, the complement potency might have been lowered sufficiently to actually affect the balance of the hemolytic system. Whenever this happens, the amboceptor titrated against the 2 units of complement employed in the test, is considerably weaker than it should be and is adjusted accordingly.

4. *The Antigens and their Daily Titrations.*—Two antigens obtained from different sources are employed: an alcoholic extract of beef-heart and a cholesterinized antigen of pig-heart. The alcoholic antigen is prepared according to Newman and Gager,[11] who recently developed this method at the Johns Hopkins Hospital. The beef-heart is first freed from fat, fiber and blood-vessels and is then ground and dried. The dried beef-heart is then extracted several times with ether, after which it is extracted with 95 per cent alcohol. The ether extract possesses little antigenic value, is rich in anticomplementary and hemolytic substances, and is discarded. The subsequent alcoholic extract, on the other hand, possesses an unusually high antigenic value with comparatively little anticomplementary and hemolytic properties.

The pig-heart antigen is prepared in the same way as the beef-heart antigen, except that it is half saturated with cholesterin, or what is equal to the same thing, 0.4 gm. of cholesterin is added to every 100 c.c. of the alcoholic antigen.

What is no less important than the preparation of the antigens is their standardization; that is, the determination of the anticomplementary and hemolytic properties and, finally, their antigenic strength or complement-binding

power. The smallest quantity of antigen, which will give a 4-plus with a known syphilitic serum, is spoken of as the antigenic unit, and because of the different properties of the two antigens employed, the respective antigenic units as used in the tests, vary widely. Thus, while the alcoholic extract antigen in present use contains from 8 to 10 antigenic units, that is, from 8 to 10 times the quantity which will bind complement with a 4-plus serum, the cholesterin antigen employed contains but 2 to 3 antigenic units or only 2 to 3 times the quantity which will give a 4-plus with a known syphilitic serum. Experience has shown that, while the alcoholic antigen possesses no tendency to pick up false positives, the cholesterin antigen possesses this property to a considerable degree. Therefore, by employing but 2 or 3 antigenic units of the cholesterin antigen in the test, it is practically impossible to lose positives and at the same time unlikely to pick up false positives. The standardization of new antigens is carried out daily for a week with different pooled positive sera, and these antigens are finally employed in the tests only after the antigenic unit is sufficiently removed from the anticomplementary and hemolytic units.

Double antigen controls are employed with each series of nine Wassermann tests. Besides this, however, in order to further insure the constancy of the binding power of the antigens, a daily titration of each antigen is run with the tests. These titrations, aside from giving the antigenic strength with the daily hemolytic system (amboceptor, complement and cells), insure also this prerequisite, that at least four times the quantity of antigen used in the test is not hemolytic and does not by itself bind complement, that is, is not anticomplementary.

With regards to fixation, it might be added that two different modes are adhered to: Ice-box fixation for a period of 4 hours, in the case of the alcoholic antigen, and water-bath fixation for ½ hour in the case of the cholesterin antigen. The superiority of the ice-box method of fixation over water-bath fixation, when the alcoholic extract antigen is employed, is well established. McNeil[12] was the first observer in this country to show the importance of ice-box fixation, and his technic is still adhered to in the New York City Department of Health. Ottenberg,[8] McNeal and Smith,[13] and others[14] have since pointed out the efficacy of this form of fixation.

The fixation of the cholesterinized antigen is carried out in the water-bath at 37.5° C. for ½ hour. The employment of ice-box fixation with cholesterinized antigen means the picking up of large numbers of false positives, as has been pointed out by a large number of workers.[13, 14] On the other hand, the fixation with this antigen for ½ hour in the water-bath, reduces this tendency to pick up false positives, to a minimum.*

5. *The Daily Control Systems.*—When the various materials which enter into the Wassermann test are prepared with the utmost care, the performance of the test itself becomes a relatively simple matter. It will be recalled that the underlying problem in the Wassermann test is to find whether some unknown serum in combination with the proper antigen will bind complement and thus prevent hemolysis of a standard amount of corpuscles in the presence of their

*Since the preparation of this paper we have been employing a fixation period of 1 hour at ice-box temperature with the cholesterinized antigen tests, with excellent results. See forthcoming issue of *Arch. of Dermat. and Syph.*

specific amboceptor (hemolysin). And, if properly controlled, no undue difficulties ought to present themselves.

The first step in the test is the standardization of the hemolytic system, that is, the finding of the proper amounts of complement and amboceptor necessary to hemolyze a standard amount of sheep's corpuscles. This being accomplished, it must be proved that the patient's serum alone, and antigen alone, do not in any way interfere with hemolysis. Then, if the patient's serum in combination with antigen prevents hemolysis, the test is positive; if they do not prevent hemolysis, the test is negative.

Not infrequently, serum and antigen will cause partial hemolysis. When this happens, the results are interpreted in accordance with the degree of hemolysis, employing the Citron[15] classification.

No Hemolysis or complete fixation = ++++
25% Hemolysis or 75% fixation = +++
50% Hemolysis or 50% fixation = ++
75% Hemolysis or 25% fixation = +
87½% Hemolysis or 12½% fixation = ±
100% Hemolysis or no fixation = −

Superimposed on all the precautionary measures mentioned in this paper, daily control systems are employed with each of the tests, which are a source of great help to us. Aside from running a 4-plus and negative control, we also run a doubtful control. As is seen from the outline, a doubtful reaction is somewhere between a one-plus and a negative, representing about 12½ per cent of complement binding; and this doubtful control is expected to show approximately the same amount of complement fixation from day to day.

The appearance of these doubtful controls often gives us a clue as to how some of the "borderline" tests are to be read. Thus, if on a given day the doubtful control appears to be more like a one-plus than doubtful, we consider our system slightly "slow", and if on that day, we are in doubt as to whether to read a given test two-plus, or three-plus, we read it two-plus. If, on the other hand, our doubtful control is very weak, we consider our system slightly "fast", and we read our borderline tests accordingly.

In conclusion, it might be stated that the Wassermann test being performed with biologic extracts and fluids, can hardly be expected to behave with the same exactness as a purely chemical test. This, in our opinion, is the very reason why no effort should be spared in rendering this test as accurate and reliable as its inherent biologic factors will permit. With every step of the test carefully controlled, it is felt that a high degree of precision is being attained. What particularly encourages us in this effort is the fact that our two Wassermann systems check practically 100 per cent.

III

WHAT THE PHYSICIAN AND HEALTH OFFICER CAN DO TO HELP INSURE CORRECT WASSERMANN TESTS

1. *The Prevention of Hemolyzed Specimens.*—Eighty specimens a month, on an average, reach this laboratory in a hemolyzed condition. Yet the waste

of time these specimens cause the physician, the patient and the laboratory can, in most cases, be prevented.

There are many reasons why blood specimens hemolyze. According to Olson[16] hemolysis is due largely to the employment of the syringe. This author claims that the force required in discharging the blood from the syringe into the vial breaks the fragile corpuscles. Roderick,[17] however, is inclined to disagree with this worker, maintaining that he has used the syringe method of blood collection in 14,500 cases with no more than 2 per cent hemolyzed specimens. It would appear that even 2 per cent is an unnecessary waste, and, if at all possible, ought to be avoided.

By far the largest number of hemolyzed specimens is caused, in our opinion, by the fact that proper clotting is interfered with. The clumping together of the red cells during coagulation tends to preserve these cells, thereby keeping the serum practically free from hemolyzed corpuscles. The more perfect the clot, the less chance there is for hemolysis. While the less perfect the clot, the greater chance for hemolysis. Recently a physician brought to the laboratory a specimen of blood which he had drawn only ten minutes before. This specimen showed marked hemolysis—undoubtedly because the blood was continuously shaken and proper clotting interfered with.

Cold retards coagulation. It is therefore best not to place the blood vial in the ice box until after the blood has coagulated. Lack of air, also, retards coagulation. This factor is partially overcome by slanting the blood vial and thus giving the blood a larger surface exposure.

It is assumed that whatever procedure is employed in obtaining blood, that sterile conditions are adhered to, and that the syringe and vial are dry and clean. If this is done and the vial slanted and permitted to remain at room temperature until clotted, the specimen ought to reach the laboratory free from hemolysis, even though it may be several days en route.

Needless to say that the best way to prevent hemolysis of Wassermann specimens is to forward corpuscle-free serum to the laboratory. This can easily be accomplished by centrifuging the blood specimen and drawing off the serum by means of a capillary pipette. When a centrifuge is not available, clear serum can be obtained by breaking up the clot with a wooden applicator and placing the specimen in the ice box for several hours. It is particularly advisable to send serum to the laboratory during hot weather when there is a greater tendency for the blood to undergo decomposition.

2. *The Prevention of Anticomplementary Specimens.*—It will be recalled that a serum is anticomplementary when it is by itself, without antigen, capable of absorbing or "fixing" unusually large amounts of complement. Thus, while an average serum, in the quantity employed in the test, will absorb from $\frac{1}{10}$ to $\frac{1}{2}$ of a unit of complement, an anticomplementary serum in the same quantity may absorb from 2 to 10 of this reagent.

Theoretically, a Wassermann test can be run with an anticomplementary specimen with reasonable accuracy. The first step is to determine the number of units of complement that the anticomplementary serum will absorb. This being accomplished, the second step is to find out how much more complement this

serum will absorb, when mixed with a standard amount of Wassermann antigen. To illustrate: If a given serum absorbs, let us say, 6 units of complement by itself and 8 or more units of complement when mixed with antigen, it is reasonably safe to consider the serum as giving a positive Wassermann reaction. The reason it is not well to run Wassermanns with anticomplementary specimens is that the results are *reasonably* correct, but not *absolutely* correct. And, until we learn more about the chemical nature of anticomplementary specimens, it is far better to obtain another specimen from the same patient and perform a regular Wassermann test.

Why are specimens anticomplementary? Frequently because of bacterial contamination. Craig[18] found that, if sera are inoculated with staphylococcus aureus and albus and other common organisms, anticomplementary properties are developed in practically all cases. This investigator further found that such contaminated specimens are actually liable to give false-positive results.

Occasionally, blood specimens which are known to be free from contamination are, nevertheless, anticomplementary. There is not, to our knowledge, a scientific explanation for this phenomenon.

When one recalls, however, that in most cases, anticomplementary specimens are due to bacterial contamination, it is evident that we are dealing with a condition which is easily preventable. Indeed, there is not sufficient reason why blood specimens should ever be contaminated, considering particularly the marked inherent germicidal properties of freshly drawn blood.

3. *Factors Influencing the Wassermann Test.*—Considering that the biology of the Wassermann test is as yet unknown, it is evident that every factor which might influence the correctness of the test should be noted. Thus, blood taken during the height of the febrile period in any of the febrile diseases will frequently give a positive Wassermann reaction in patients apparently free from syphilis.[19] The ingestion of alcohol appears to have an opposite effect. Within twenty-four hours after taking some alcohol, positive reactions will frequently become negative. This, it appears, does not apply to gin. Indeed, it is claimed that within twenty-four hours after the intake of gin, a normal individual will occasionally give a positive Wassermann reaction (Wile). Blood for a Wassermann test taken during anesthesia will also frequently give a false-positive reaction.

Among the nonsyphilitic diseases which have been reported to give a positive Wassermann reaction, are frambesia (yaws), leprosy, the febrile stage of malaria, relapsing fever, yellow fever, and occasionally pellagra. Fortunately, with the exception of malaria, most of these diseases are quite uncommon and, therefore, play but a little part in influencing the interpretation of the test.

4. *The Handling of Wassermann Specimens.*—The physician, who sends specimens to the laboratory to be tested, has a right to expect that they will be looked after and examined with the greatest care. Has not the laboratory the same right to expect similar care on the part of the physician in the proper taking and forwarding of specimens?

Blood for Wassermann tests reaches this laboratory in bottles of nearly every conceivable type. It is assumed, of course, that in practically all cases

they have been properly washed and sterilized before using. The occasional container, however, which is hastily washed, may hold chemical substances that might influence the correctness of the Wassermann test. In order to overcome just such conditions, this Health Department Laboratory, as well as similar laboratories, furnishes sterile vials upon request, and as far as possible only these vials should be used.

Health officers who are called upon to obtain specimens of blood in large numbers, as is frequently true in testing food-handlers, are particularly urged to see that those who record the names are sufficiently responsible to recognize the great danger of confusing specimens. An error of this kind recently came to our attention and would have worked serious harm, were it not for the fact that the health officer traced and corrected it in time. There is but one way to overcome such errors, namely, that all those who are in any way connected with Wassermann specimens must be taught the importance of handling such specimens with the utmost care.

IV

THE INTERPRETATION OF WASSERMANN RESULTS

1. *Interpreting the Laboratory Report.*—Some laboratories report results of the Wassermann test as positive, doubtful and negative. Other laboratories report Wassermann results as two-plus (++), one-plus (+), doubtful (±), and negative (−). Still others report four-plus (++++), three-plus (+++), two-plus (++), one-plus (+), doubtful (±), and negative (−). This last mode of reporting as proposed by Citron is adhered to in this laboratory.

A four-plus (++++) report means that the Wassermann test is 100 per cent positive; a three-plus (+++), that it is 75 per cent positive. A two-plus (++) report means that the test is 50 per cent positive; a one-plus (+), that it is 25 per cent positive; and a plus-minus (±), that it is from 10 to 15 per cent positive. We adhere to this mode of reporting, because in our opinion it is of greater help to the physician in interpreting his clinical findings.

We are frequently asked to interpret the meaning of a doubtful-positive or plus-minus (±) report. A doubtful-positive is the weakest positive reported, ranging as was stated above, from a 10 to a 15 per cent positive Wassermann test. In the absence of clinical history and findings, such a report could undoubtedly be disregarded. In the presence of clinical manifestations, however, a doubtful report should be considered as a weak-positive reaction.

The Wassermann tests performed with the cholesterinized antigen show a tendency of "picking up" doubtful positives, even in normal individuals. The tests performed with the alcoholic antigen do not appear to possess this property. Under these conditions, when a Wassermann test is reported doubtful-positive (±) with the cholesterinized antigen alone, it is far less significant than when the test is reported doubtful-positive (±) with both the alcoholic and cholesterinized antigens, or even with the alcoholic antigen alone.

When the clinical manifestations on the part of the patient are of a doubtful nature and the Wassermann report is doubtful positive (±), it is well to repeat the Wassermann test two or three times at about 10-day intervals. The

repeated tests may be negative, doubtful-positive (±), or one-plus (+) or two-plus (++). The clinician will thus be able to judge accordingly. Should the tests continue to be doubtful-positive (±), the institution of antisyphilitic treatment must be based on a more thorough search of clinical manifestations.

2. *In Untreated Cases of Syphilis.*—We are accustomed to think that a patient suffering from syphilis has *syphilitic antibodies* in his blood and that the Wassermann test proves the presence or absence of these antibodies. This hypothesis renders the interpretation of Wassermann results quite difficult. We know that in most infections, antibodies circulate in the blood long after the disappearance of the infective agent from the body, while in syphilis, just as soon as the *spirochete* are exterminated from the body, the Wassermann test to our knowledge becomes negative. What is probably more likely, is that the Wassermann test is an index to the presence in the system of the *Spirochete pallida* or some product of these organisms, which some writers speak of as syphilitic reagin. And with this view in mind, the interepretation of the Wassermann test in the various stages of syphilis is relatively simple.

In the early primary stage, when the amount of syphilitic reagin circulating in the blood is comparatively small, the Wassermann reaction is likely to be negative or very weakly positive. In the later primary stage as the reagin increases in the system, the Wassermann reaction usually becomes stronger; while in the still later primary stage, still stronger. Craig, who has made a study of the appearance of the Wassermann reaction in 600 cases of primary syphilis, found 36 per cent positive reactions during the first week after the appearance of the chancre; 59 per cent during the second week; 68 per cent during the third week; 77 per cent during the fourth week; and 81 per cent during the fifth week.

It is evident that a negative Wassermann result during the primary stage does not exclude the possibility of syphilis. Furthermore, a one-plus (+) or plus-minus (±) reaction in this stage might safely be considered as sufficient cause for specific treatment. When the Wassermann is negative in the primary stage, we usually recommend that another blood specimen be forwarded for reexamination in a week or ten days.

In untreated cases of secondary syphilis the Wassermann test is positive in from 92 to 100 per cent of cases. The intensity of the reaction, however, does not always correspond to the severity of the infection, although, as a rule, the more severe the infection, the stronger the Wassermann reaction. It is well to point out in this connection that precocious malignant syphilis, which is perhaps one of the worst manifestations of this disease, gives, as a rule, a negative Wassermann reaction (Wile).

The Wassermann test in tertiary syphilis is positive in about 96 per cent of cases. This test is particularly valuable in this stage of the disease, since the clinical symptoms are, in many cases, obscure. In this condition, Wassermann tests on the spinal fluid are often of great value, particularly when the blood gives a weak or negative reaction.

In latent syphilis where the *spirochete* are dormant and little tissue destruction is going on, one finds a smaller per cent of positives as compared with more

active syphilitic conditions. Craig reports 67 per cent of positive reactions in latent syphilis. Due to the dormant condition of the organisms, one also is likely to find numerous weak reactions in this stage.

In this form of syphilis, particularly in the presence of nervous symptoms, the Wassermann reaction of the spinal fluid is frequently positive when the blood reaction is negative. It is important, therefore, to determine the Wassermann reaction of this fluid in all cases of suspected latent syphilis which give negative reactions in the blood. A positive reaction in the spinal fluid often establishes a diagnosis of neurosyphilis long before nervous manifestations set in.

In congenital syphilis the Wassermann test shows a high per cent of positives. Children showing syphilitic lesions at birth usually give positive Wassermanns in 100 per cent of cases. Where the lesions appear later in life, the Wassermann test, it is claimed, misses occasional cases of this form of syphilis.

It is evident from the foregoing that in every syphilitic stage isolated cases are found which will not respond to the Wassermann test. Syphilologists insist, therefore, that in the presence of clinical symptoms a negative Wassermann test does not exclude syphilis.

3. *In Cases Undergoing Treatment.*—There is much difference of opinion as to the interpretation of the Wassermann test in treated cases. According to Wile and Hasley,[20] a patient who has undergone intensive treatment and is clinically free from the disease may, with reasonable safety, be considered cured, even though his blood gives a positive Wassermann reaction. "We are convinced," say these investigators, "that in the presence of an intensive therapy, a positive test does not necessarily mean living spirochetes and potential syphilis any more than a positive tuberculin test in an individual who has had tuberculosis would indicate the presence of living tubercle bacilli." This view is shared by numerous syphilologists.[21] There are others,[22] however, who insist that a patient cannot be considered cured unless he gives a negative Wassermann reaction. In summing up this subject, Kolmer[23] states, "It has been abundantly proved, however, that in syphilis a single negative reaction is not sufficient or definite evidence that a cure has been effected, for the disease may recur after treatment is discontinued, at least to the extent that the Wassermann reaction reappears, followed by clinical manifestations. It is necessary, therefore, that successive examinations be made during a period of at least two years, and off and on during the remainder of life." It appears that Wile and his school, particularly object to the so-called "serologic cure," i. e., the employment of the Wassermann test as the criterion as to whether or not a patient is cured. The biology of the Wassermann test being unknown, they insist that the clinical condition of the patient must be of first consideration and the Wassermann results of secondary consideration, in attempting to judge the results of treatment. To these workers, the presence of a positive Wassermann reaction implies that a patient has or has had syphilis.

It would seem that each case represents its own individual problem. And the consensus of opinion among syphilologists appears to be that in the general run of cases, treatment should be continued until the Wassermann is

negative. In the so-called "Wassermann fast" cases, however, where the Wassermann reaction is persistently 4+, the primary aim should be to clear up the clinical symptoms and to institute further treatment only after considerable periods of rest.*

We are frequently asked how long after the administration of antisyphilitic treatment should one attempt to determine the value of the treatment by means of the Wassermann test. For two or three weeks following treatment, the Wassermann reaction becomes weak or negative in most cases. Frequently, however, this is but a temporary manifestation due to the immediate effect of treatment. If the Wassermann reaction is negative two months or longer after treatment, it is reasonably safe to assume that the treatment had its desired effect. Needless to say that the retesting of blood of supposedly cured cases, let us say, at six-month or yearly intervals should be encouraged in all cases.

4. *The Provocative Wassermann.*—Occasionally the administration of antisyphilitic treatment, such as mercury or salvarsan, will in the course of about three days change the Wassermann reaction of a syphilitic patient from negative to positive. This is known as a *Provocative Wassermann Reaction* and is claimed to occur only in latent cases of syphilis. The explanation of this reaction is not quite clear. The antisyphilitic treatment possibly awakens the dormant spirochete into greater activity, thus causing them to liberate sufficient *reagin* to render the test positive. There is, however, a tendency on the part of syphilologists to place less and less faith in the provocative Wassermann reaction.[24] Some workers believe, for example, that the administration of salvarsan or neosalvarsan will, in some cases, bring about a positive Wassermann reaction even in nonsyphilitic conditions. Strickler, Munson and Sidlick[25] recently reported a series of cases which developed positive Wassermann reactions after administration of salvarsan, although, in their opinion, syphilis could absolutely be excluded from them.

With regard to the spinal fluid, Solomon and Klauder[26] report in a recent paper that the intravenous or intraspinal injection of arsphenamine changed negative to positive Wassermann reactions in a number of syphilitic cases, no provocative reaction being obtained with the blood serum in these cases. These workers, however, do not claim this to be a frequent phenomenon. Vascular neurosyphilis, for example, with negative cerebrospinal fluid frequently does not react in this manner.

The consensus of opinion of syphilologists appears to be that the development of a positive reaction following salvarsan and neosalvarsan treatment should be interpreted with due caution and in conjunction with the clinical evidence of the case.

5. *The Wassermann Test in Pregnancy.*—Prof. J. W. Williams[27] of Johns Hopkins University has recently completed some studies on the significance of the Wassermann test in obstetrics, which throws much light on this highly im-

*It must be remembered that the designation of 4+ is arbitrary; some syphilitic sera, as determined by special procedure, are as strongly positive as 40+. A persistent 4+, therefore, does not necessarily imply that the antisyphilitic treatment is having no effect on the Wassermann reaction. A syphilitic serum may be 36+ before treatment; 20+ after the first course and 6+ after the second course of treatment, although the regular laboratory report would be 4+ in each of these examinations.

portant problem. For the past several years, a Wassermann reaction was determined in every pregnant woman who registered in the Dispensary of Johns Hopkins Hospital, and, if it was found to be positive, the patient, whenever possible, was subjected to antisyphilitic treatment. Of 421 women who gave positive Wassermann reactions on admission, 157 did not receive any treatment (group a); 103 received inefficient treatment (group b); and 163 received satisfactory treatment (group c). The result of treatment is shown by the fact that 52 per cent of children of *group a* were born dead or presented some clinical evidence of syphilis, as compared with 37 per cent in *group b* and 7 per cent in *group c*. To quote Professor Williams, "The evidence at our disposal shows that, if syphilis is recognized early in the pregnant woman and is intensively and appropriately treated, almost ideal results may be obtained, so far as the child is concerned."

It occasionally happens that a woman will give a positive Wassermann reaction during pregnancy; give birth to a syphilitic child; and in some weeks after childbirth, give a negative Wassermann reaction. There is no satisfactory explanation for this phenomenon. Neither is there an explanation for the fact that a pregnant woman will in some instances give a negative Wassermann reaction, and yet give birth to a syphilitic child.

6. *A Positive Wassermann in the Absence of Clinical Evidence.*—In the early history of the Wassermann test, numerous workers reported positive reactions in many conditions other than syphilis. In carcinoma, for example, as well as in diabetes, the Wassermann test has been reported positive by various workers. These reports, we believe, were due to the fact that in the hands of early workers the technic of the Wassermann test was not sufficiently fine.

One still occasionally hears that a positive reaction is obtained in scarlet fever. Recent work, however, appears to disprove this. Kolmer, for example, tested the blood of 250 cases of scarlet fever with only 2 per cent of positive results and in this small number syphilis could not be excluded. As pointed out above, one of the important nonsyphilitic diseases which gives a positive Wassermann reaction is frambesia (yaws) which is caused by an organism morphologically similar to the Spirocheta pallidum (Spirocheta pertenuis). Positive reactions have also been reported in leprosy, in the febrile stage of malaria, in relapsing fever, and occasionally in pellagra. When these conditions are eliminated, a 4-plus reaction should be considered a symptom of syphilis even in the absence of clinical evidence.

In this connection, it may be worth while to give the unanimous report of the Committee on Diagnosis and Treatment of Syphilis of the All American Conference on Venereal Diseases held in Washington in December, 1920, under the auspices of Dr. C. C. Pierce, Ass't Surgeon General, U. S. Public Health Service. (Members present: Drs. Wm. Edler, H. H. Hazen, Wm. A. Pusey, W. Wende, J. S. Salas and A. B. Vasconcelos.)

Question: To what extent should a positive Wassermann be relied upon as evidence of syphilis in the absence of clinical symptoms and signs?

Answer: That a frank, reliable Wassermann should be regarded as a symptom of syphilis.

Caution: (a) In the absence of all other evidences of syphilis, a diagnosis of syphilis upon a positive Wassermann reaction alone should be made with great caution.

(b) In the absence of all other evidences of syphilis, the Wassermann result should be verified by several repetitions at the hands of different observers, if possible. The presence of other conditions which might cause a positive Wassermann should be excluded as far as possible.

(c) There should be a very careful search for other evidences of syphilis, including clinical, serologic, pathologic, and other examinations.

Conclusion: After all of the above conditions have been complied with, a positive Wassermann should be assumed to be evidence of the existence of syphilis.

Needless to say that weak-positive reactions should be fully correlated with the clinical signs before reaching a positive diagnosis. The blood of normal individuals will occasionally give a one-plus (+) or plus-minus (±), particularly with a cholesterinized antigen. These weak reactions, in the absence of clinical symptoms or history, could undoubtedly be considered negative.

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TWO STAINS USED IN PREFERENCE TO WRIGHT'S STAIN IN THE ROUTINE STAINING OF BLOOD SMEARS

By Garnet B. Grant, B.S., M.D., and Eric R. Wilson, M.A., Los Angeles, Cal.

WE have found that the following stains used in the routine staining of blood smears for differential counts are much more satisfactory than Wright's, Jenner's, or any of the other more common stains. Moreover the smear does not require preliminary fixation.

Solution 1. Sat. sol. eosin in methyl alcohol
Solution 2. Mallory's instantaneous hematoxylin

Technic.—Smear should be even and not too heavy.
Dry in air or pass through flame.
Stain with solution Sat. sol. methyl eosin, (sol. No. 1) 2 to 2½ minutes.
Wash in distilled water.
Stain with sol. No. 2 hematoxylin 3 to 4 minutes.
Wash in distilled water, blot and dry.

This stain will be found entirely satisfactory for differential counts, but is unsatisfactory for staining malarial parasites, and on this account have adopted the use of the following stain as a routine on all smears suspected of malaria.

Solution 1. Sat. sol. eosin in methyl alcohol
Solution 2. 0.25% aqueous sol. azure 11

Technic.—Smear should be even and not too heavy.
Dry in air or pass through flame.
Stain with solution No. 1, 2 to 2½ minutes. (methyl eosin.)
Wash in distilled water.
Stain with sol. No. 2 (Aqueous Azure 11) 20 to 40 seconds
Wash in distilled water—blot—dry.

While this latter makes a most beautiful stain and is well adapted for all routine work including the staining of the malarial parasites, in unskilled hands there will be found a tendency to overstain with the second solution (Azure 11).

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EDITORIALS

Diseases of Animals Communicable to Man

HOBDAY[1] gives the following list of diseases among animals in Great Britain which are communicable to man: Glanders, anthrax, tuberculosis, rabies, foot and mouth disease, mange of all animals (horse, ox, camel, dog, and cat), certain forms of seborrhea, and pyorrhea. Hobday says that pyorrhea is very prevalent in pet dogs and he thinks it highly possible that it may be transmitted from dogs to men or in reverse order. Of course, certain parasitic diseases in addition to those above mentioned, such as echinococcus, tænia, and trichina, are also transmissible from the lower animals to man; in fact, residence in the two hosts is necessary in order to complete the life cycle of these parasites.

Through the aid of the mallein test and the destruction of glandered animals, glanders in Great Britain has decreased almost to the vanishing point. In 1901, 2,370 horses were destroyed on account of glanders in Great Britain, and 1,828 of these were found in London. The veterinary department of the Ministry of Agriculture shows that during the year 1920 only fifteen outbreaks of this disease occurred in the whole of the British Isles, with a total

[1]Lancet, 1921, cc, 276.

destruction of only twenty-two animals, and it is well to remember that these occurred after the sale and distribution of more than 150,000 army horses and mules gathered together from various countries and employed in the war. Twenty years ago it was a daily occurrence for a veterinarian to be called to one of the municipal stables in London to inspect the horses and within one year in one stable of 2,000 horses ninety glandered ones were found. At the present time the use of mallein and its repeated use is compulsory. When an outbreak of this disease is reported in any stable every animal in the stable is quarantined and tested. All ponies sent into the mines and all horses and mules used for special purposes are given the mallein test. During the war special caution was taken to isolate and test all purchased animals. All units were malleined at regular intervals and each individual animal was tested before being sold, even though its destination was the slaughter-house and its flesh was to be served as food for man. Hobday states that in the whole allied forces there was no army whose animals were so free from glanders as the British. It seems quite certain that glanders will soon be unknown in the British Isles.

During the year 1919, 180 outbreaks of infection with anthrax occurred in England and Scotland. It seems that Wales was absolutely free from this disease and had been for three years previously. In all, 275 cattle, eight horses, one sheep, thirty-eight pigs, one dog, and seven ferrets, were affected. Twenty-five per cent of the outbreaks occurred on premises where anthrax had been in evidence during the previous year. The veterinary minister states that the number of cases of anthrax in animals has been reduced from about 1,000 in 1910 to less than 300 in 1919. The sources of infection from anthrax in Great Britain are: (Effluents from tanneries passing into streams. (2) Feeding infected offal to pigs. (3) Use of imported foodstuffs and artificial manures. (4) Contaminated sewage. (5) Infected brushes and furs.

For twenty years before the War, there had been no rabies in England. This was due to the close supervision and quarantining of dogs imported into that country. The first case was brought into Plymouth during the month of May, 1918, and during 1919 there were 143 cases of rabies in England among animals, 140 in dogs, two in horses, and one in a pig.

Great Britain was free from foot and mouth disease from 1895 to 1899 and again from 1903 to 1908. During 1920 there was an outbreak of obscure origin. When we remember what great destruction foot and mouth disease has caused in other countries, we can understand the desirability of excluding it. In 1919, northern Italy was visited by a very virulent form of this disease and many of the farmers were ruined in consequence of it.

There are three varieties of parasites that cause mange in domestic animals, and this disease is widely distributed. In the horse, or rather on the horse, the three varieties embrace a sarcopt, a psoropt, and a symbiot, but the chief trouble is due to the two first mentioned and they are transferred to man, especially to individuals engaged in feeding and grooming horses. Mange as transferred from horse to man most frequently infects the surface of the forearm and the hands. Man may become infected by riding mangy horses. During the war this infection frequently occurred, and in these cases

the disease was in evidence on the inside of the legs and in the pubic region. Persons engaged in skinning mangy horses are likely to be infected. Sarcoptic mange is not easily got rid of on horses and requires a treatment which may extend through several months. The psoroptic variety is much more amenable to treatment. In Great Britain mange is a notifiable disease. Sulphur in some form or another forms the basis of most treatments. Hobday says that mange in the dog and cat is transmissible to man, and Whitfield has recently reported seventeen cases in which he confirmed the existence of the sarcoptic parasite in man and traced it to the dog. Owners of mangy dogs often complain of an irritable skin, usually on the inside of the forearm where the dog rests its head while being petted. The warm flesh of the man causes the parasite to leave its canine host and take up its residence on human tissue where it is capable of producing marked irritation. In one of the seventeen cases reported by Whitfield the infected region was on the neck and chest where the owner was in the habit of allowing his pet dog to lie. The lesions are described by Whitfield as small vesicles surrounded by narrow zones of hyperemia, very like that of varicella, but only about one-eighth the size. These lesions are scattered discretely over the surface and are not in groups. If left untreated this parasite may live on man for about six weeks, but under proper sulphur treatment it is easily got rid of. Mangy cats, especially male animals, wander widely and spread their parasites freely.

In the country calves, and in the city cats, are chiefly instrumental in the distribution of ringworm. This infection is often quite resistant to treatment. Hobday complains because there is no adequate meat inspection in England, and consequently measly meat is frequently marketed. Fortunately, the Englishman is not given to eating raw meat and parasites of this nature are generally killed in the process of cooking.

—*V. C. V.*